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TNT Metabolites in Animal Tissues
Final Report

December 1990

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Supported by

U.S. ARMY MEDICAL RESEARCH AND
DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21702-5012
Army Project Order No. 88PP8866

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Oak Ridge, Tennessee 37831-6036

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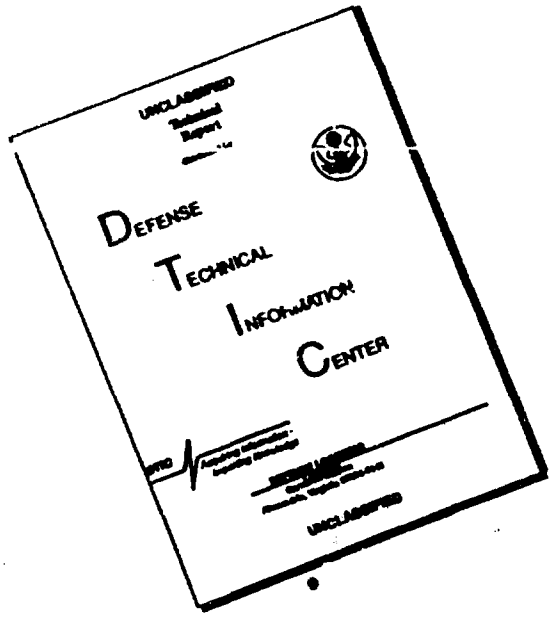

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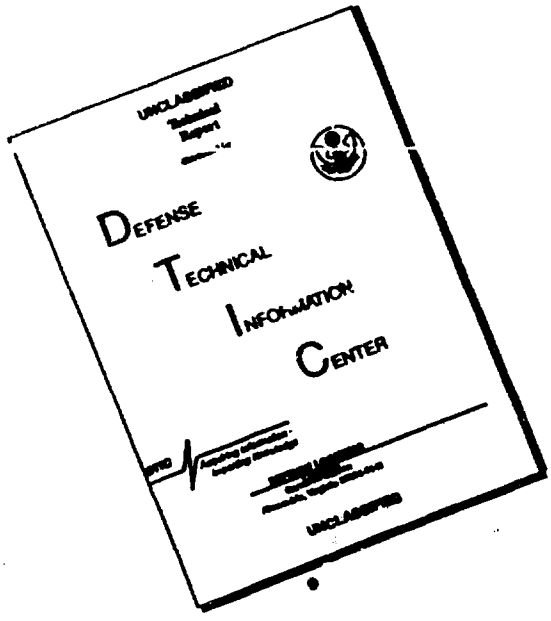

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Environmental Sciences Division
Publication No. 3718

Prepared by the
Environmental Sciences Division
OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37831-6285
managed by
MARTIN MARIETTA ENERGY SYSTEMS, INC.
for the
U.S. DEPARTMENT OF ENERGY
under contract DE-AC05-84OR21400

ORNL/M-1336

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT: Approved for Public Release
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	Distribution Unlimited
4. PERFORMING ORGANIZATION REPORT NUMBER(S): ORNL/M-1336	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Oak Ridge National Laboratory; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Environmental Sciences Division, PO Box 2008, Oak Ridge, TN 37831-6036	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION: US Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Army Project Order No. 88PP8866
8c. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21702-5012	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO. 3E1-	TASK NO.	WORK UNIT ACCESSION NO
0602720A	62720A835	00	WUDA315688

11. TITLE (Include Security Classification): TNT METABOLITES IN ANIMAL TISSUES

12. PERSONAL AUTHOR(S): L. R. Shugart and W. H. Griest

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
FINAL	FROM 9/30/88 TO 9/30/90	1990 December 15	47 + Appendices

16. SUPPLEMENTARY NOTATION

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Trinitrotoluene; TNT, TNT-related Metabolies; Deer, Quail and Rabbit; HPLC Analysis; Statistical Design; Decision Tree Analysis; Environmental Contamination; RA 3

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Analyses for TNT and nine potential metabolites (TNT-related compounds) were made in deer, rabbit, and quail tissues (muscle and liver) taken from the Alabama Army Ammunition Plant (AAAP), Childersburg, Alabama. The listed TNT-related compounds are 2,4,6-trinitrotoluene (parent compound); 2,4-diamino-6-nitrotoluene; 2,6-diamino-4-nitrotoluene; 2-amino-4,6-dinitrotoluene; 4-amino-2,6-dinitrotoluene; 2,4,6-trinitrobenzyl alcohol; 2,4,6-trinitrobenzoic acid; 1,3,5-trinitrobenzene; 4-hydroxylamino-2,6-dinitrotoluene; and 2,6,2',6'-tetranitro-4,4'-azoxytoluene. The procedure for extraction of these compounds from animal tissue required homogenization in acetonitrile, and subsequent partitioning into chloroform. Quantitative determination of extracted compounds was obtained by chromatographic separation on a mixed-mode HPLC column in which the phase bonded to the silica surface contained both a C18 (reversed-phase function) and a secondary amine (anion exchange function) incorporated into a single ligand. A ternary mobile phase gradient containing pH 5.1 phosphate buffer, methanol, and acetonitrile was used in the separation. Detection was by UV-absorbance at 254 nm and the practical

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Mary Frances Bostian	(301) 663-7325	SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE: UNCLASSIFIED

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Item 19.

ABSTRACT (continued)

detection limit for each compound was 0.2 micrograms per kilogram (PPM) of tissue. No TNT-related compounds were found at or above the detection limit in the tissues of twelve deer, five rabbits, and five quails taken from the AAAP. The U.S. Army Biomedical Research and Development Laboratory provided a study design criterion level that the total concentration of TNT and related metabolites should not exceed 1.2 mg per kg of tissue. Accordingly, a statistical approach was designed to provide confidence statements concerning the presence of criterion levels in the animal population when the compounds were not detected in sample tissues. Application of this approach on the data collected on deer tissue from the AAAP site suggest that, with 95% confidence, no more than 65 animals from a total population estimated to be 300 have TNT-related compounds in their tissues that exceed 0.2 PPM (detection limit), and further, that no more than 0.26% of the 300 animals would have these compounds at a concentration that exceed the study design criterion level. In essence the significance of the statistical analysis is that the deer population at the AAAP site do not have TNT-related compounds in their tissues that exceed the criterion level.

An experimental verification of the metabolism of TNT and the detection (or absence) of the selected metabolites was performed in mice subacutely dosed with 100 milligrams per kilogram of [^{14}C]-TNT. These studies show that the TNT-related compounds of concern do accumulate in muscle and liver tissue of the mouse under the experimental conditions imposed, but at concentrations below the 1.2 PPM level. However, products other than TNT and free metabolites may be accumulating since some [^{14}C] was found to be nonextractable.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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Lee Shugart December 15, 1990
PI Signature Date

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I. INTRODUCTION

1. Background

Regulatory agencies have recommended that tissue of deer living in TNT-contaminated areas of the former Alabama Army Ammunition Plant (AAAP) site at Childersburg, AL be analyzed to ensure that they are free of excessive toxic contamination resulting from ingestion of contaminated soil, water and plant materials. The potential compounds of concern are the parent compound 2,4,6-trinitrotoluene (TNT); 2,4-diamino-6-nitrotoluene (2,4-DA-6-NT); 2,6-diamino-4-nitrotoluene (2,6-DA-4-NT); 2-amino-4,6-dinitrotoluene (2A-4,6-DNT); 4-amino-2,6-dinitrotoluene (4A-2,6-DNT); 2,4,6-trinitrobenzyl alcohol (TNB alcohol); 2,4,6-trinitrobenzoic acid (TNB acid); 1,3,5-trinitrobenzene (TNB); 4-hydroxylamino-2,6-dinitrotoluene (4HOA-2,6-DNT); 2-hydroxylamino-4,6-dinitrotoluene (2HOA-4,6-DNT); and 2,6,2',6'-tetranitro-4,4'-azoxytoluene (Azoxydimer). These compounds were identified (El-hawari et al., 1981) in the urine of various small animals after administration of TNT. The chemical structure and possible route of metabolic formation are detailed in Figure 1. The study design criterion level for total concentration of TNT and metabolites had been established as 1.2 mg/kg in animal flesh (Rosenblatt, personal communication). In setting this level, Rosenblatt assumed that all involved compounds were comparable in toxicity to TNT and used plausible estimates of the average daily intake of venison by exposed individuals. Thus, the design analytical sensitivity goal for any of the individual compounds was 0.1 milligrams per kilogram of flesh.

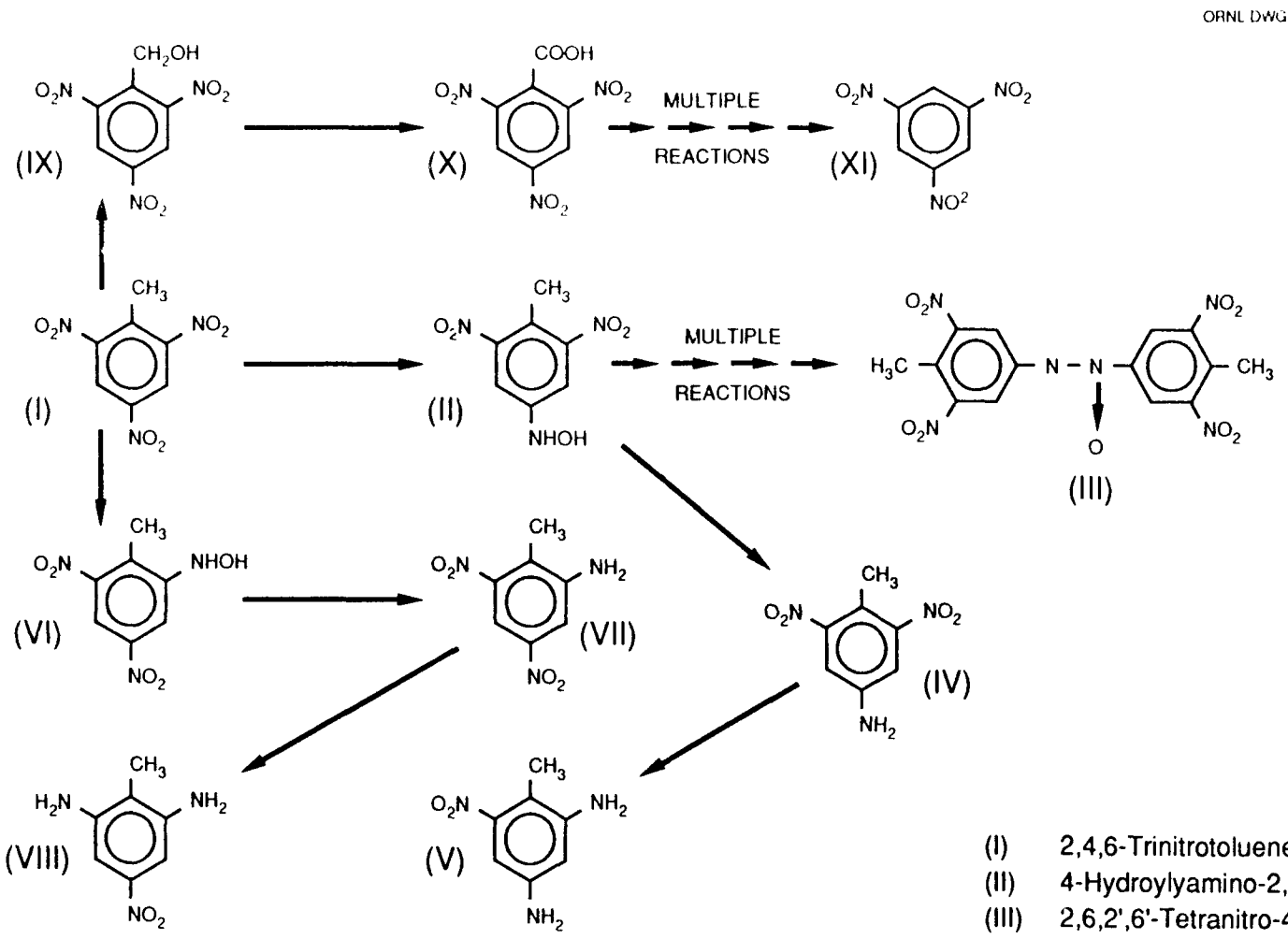


Figure 1: Schematic presentation for some possible biotransformation products of 2,4,6-TNT

(I) 2,4,6-Trinitrotoluene
(II) 4-Hydroylyamino-2,6,dinitrotoluene
(III) 2,6,2',6'-Tetranitro-4,4'-azoxytoluene
(IV) 4-Amino-2,6-dinitrotoluene
(V) 2,4-Diamino-6-nitrotoluene
(VI) 2-Hydroxylamino-4,6-dinitrotoluene
(VII) 2-Amino-4,6-dinitrotoluene
(VIII) 2,6-Diamino-4-Nitrotoluene
(IX) 2,4,6-Trinitrobenzylalcohol
(X) Tinitrobenzoic acid
(XI) Triniotrobenzene

2. Project Aims

The overall objectives of this study were to: (1) provide quantitative analytical procedures for the analysis of TNT and related metabolites at a level of 0.1 milligram per kilogram of tissue or less for each compound; and (2) obtain representative samples of tissues from several species of animals (deer, rabbit, and quail) at the AAAP site and to determine the presence or absence of TNT and its metabolites in these samples.

3. Experimental Approach

To meet the overall objectives of this project, the following individual tasks were identified:

Task 1 - Obtain suitable amounts (0.1 to 1.0 grams) of the parent compound (TNT) and metabolites either from commercial sources or by chemical synthesis.

Task 2 - Develop analytical procedures for the detection and quantification of the TNT and related compounds.

Task 3 - Demonstrate the extractability of all the TNT-compounds from "spiked" animal tissue at an analytical sensitivity of 0.1 mg/kg/metabolite and attempt to verify the methodology by separating and quantifying the TNT-compounds in laboratory animals (mouse or rat) dosed subacutely with TNT.

Task 4 - Obtain tissue from deer, rabbit, and quail at the AAAP site in Childersburg, AL and analyze each sample for TNT-compounds following procedures developed in Tasks 1, 2, and 3. Present a Statistical Design for sampling and analysis of deer tissue.

II. EXPERIMENTAL METHODS

1. Procurement/Chemical Synthesis of Authentic Compounds

Ten TNT metabolites were either purchased or synthesized. Seven compounds were purchased from Aldrich Chemical Co., Milwaukee, WI: TNT; TNB acid; 2A-4,6-DNT; 4A-2,6-DNT; 2,4-DA-6-NT; 2,6-DA-4-NT; and TNB. The purity of each compound was checked using high-performance liquid chromatography (HPLC) on a C-18 reversed phase column. With the exception of TNB acid, the purity of each purchased compound was found to be more than 99%.

The commercially-available TNB acid was only 60-70% pure. Further purification was performed using HPLC, and the structure of the product was deduced from both its positive acidic character (extractable by sodium hydroxide solution) and its infra-red spectrum (carboxyl group plainly visible).

The 4HOA-2,6-DNT was synthesized according to Nielsen et al (1979). It was purified using a combination of preparative scale silica column, and preparative TLC. The structural determination was based upon mass spectrometry. About 100 mg of pure compound was prepared.

The azoxydimer was synthesized easily according to Sitzmann (1974). The product was recrystallized twice from benzene, yielding 50 mg of purified compound.

While the TNB alcohol may be prepared using several different reaction methods, the best is that reported by Gauguly (1925). A modified synthesis procedure consisting of a two-step reaction with an overall yield of 12%, is described as follows:

(i) Bromination of TNT with liquid bromine at 150-160 °C for 20 hours yields 20% 2,4,6-trinitrobenzyl bromide.

(ii) Hydrolysis of 2,4,6-trinitrobenzyl bromide at 115 °C for three hours yields 60% TNB alcohol.

Both the intermediate and final products were purified by HPLC, and the bromination and hydrolysis, respectively, were confirmed by mass spectrometry. About 100 mg of purified TNB alcohol was obtained.

[^{14}C]TNT at a specific activity of 21.6 millicurie per millimole was obtained from ChemSyn Science Lab., Lenexa, KS. A purity of >98% was verified by HPLC. A validated method for synthesis of 2HOA-4,6-DNT could not be found; see Section 4 in the DISCUSSION/CONCLUSION section for more details.

2. Procedures for the Analytical Detection of TNT and Metabolites

Previous workers (e.g., Kaplan and Kaplan 1982; Yinon and Hwang 1985; and Elhawari et al., 1981) have used both reverse phase and normal phase HPLC to separate TNT and certain of its metabolites. We tested these and other reverse phase HPLC methods but were not able to satisfactorily resolve all the compounds required for this study. A separation and detection procedure for TNT and its possible metabolites was successfully developed using a mixed-mode HPLC. The HPLC column (Alltech Associates, Inc., 150 mm X 4.6 mm i.d.;

5 micron particle size; Cat. No. 72620) stationary phase bonded to the silica surface contains both a C18, (reversed-phase function) and a secondary amine, (anion exchange function) incorporated into a single ligand in a 1:1 ratio. The separation utilizes a gradient built from three different eluting solvents: A. an aqueous solution containing phosphate that has been adjusted to pH 5.1 with the final concentration of phosphate being 0.015 M in a 10:90 water:methanol solvent; B. methanol; and C. acetonitrile. The sources of phosphate are potassium dihydrogen phosphate and dipotassium hydrogen phosphate in the appropriate amounts to achieve the desired pH and concentration. Control of pH with the buffer is crucial to achieving the necessary separation. The gradient program starts with equilibration at 72% Solvent A and 28% acetonitrile and elution with this mixture for 1 min after sample injection. From 1 to 5 min a linear gradient reduces solvent A from 72% to 68% and increases acetonitrile from 28% to 32%. Isocratic elution conditions are then maintained for the time interval from 5 to 14 minutes. Between 14 and 20 min a linear gradient reduces solvent A by 4% per minute and increases both methanol and acetonitrile by 2% per minute so that at 20 min the eluting solution is 44% solvent A, 12% methanol and 44% acetonitrile. From 20 to 26 min a linear gradient reduces solvent A from 44% to 2% and increases methanol from 12% to 54% while maintaining the acetonitrile at 44%. Isocratic conditions are then maintained from 26 to 33 min. Then between 33 and 38 min a very steep gradient restores the eluting solution to its starting conditions after which the column is re-equilibrated for 7 min.

For analysis, a 50-μL aliquot of a sample is injected onto the column maintained at ambient temperature and eluted at a flow rate of 1 mL per min. Detection is at 254 nm with a Hewlett-Packard 1090 Liquid Chromatograph equipped with a diode array UV detection, automatic sampling system, and Chem Station data system. The instrument was calibrated using eight different concentrations of TNT and metabolites over the range of 0.1 through 5 PPM in solution.

3. Extraction Procedure for the Recovery of TNT and Metabolites from Animal Tissue

An extraction procedure for the isolation and recovery of TNT and metabolites from animal tissue was developed using samples spiked with known amounts of TNT-related compounds. The procedure was as follows:

(i) Thaw tissue (1.96 - 2.04 g) on ice and mince. Transfer to centrifuge tube.

(ii) Add 6 mLs of CH_3CN (note: all organic solvents are pretreated with Na_2SO_4 before use) and homogenize with a "Polytron" (3X, 200 watts, ~15 sec).

(iii) Remove debris by centrifugation (Sorvall Superspeed RC2-b; 10 min, 0°C; 3000 rpm) and rewash pellet with 6 mLs CH_3CN, repeat centrifugation, combine supernatants, and take to dryness with N_2.

(iv) Resuspend dried extract in 2 mLs H_2O by shaking for 1 min then transfer to separatory funnel. Add 5 mLs $CHCl_3$ and mix phases by shaking (50X). Recover $CHCl_3$ phase and re-extract H_2O phase with 5 mLs $CHCl_3$. Combine CH_3Cl phases, add 1 g of Na_2SO_4 and mix by vortexing. Recover $CHCl_3$, take to dryness with N_2, resuspend in 100 μL CH_3OH, and store at 4°C.

4. Dosing of Mice with [^{14}C]TNT

Young adult mice (Swiss Webster outbred strain) of both sexes weighing approximately 30 grams each were divided into three groups of four animals in each group and placed in metabolic cages. Each animal in groups one and two was administered 100 milligrams per kilogram body weight of [^{14}C]TNT (Sp. Act. 21.6 mCi/mmole) in corn oil by gavage in four equal doses over a period of one week. The third group received corn oil only. Two days after receiving the final treatment, the animals were anesthetized and ~1 milliliter of blood was withdrawn from the heart of each animal. In addition to the blood, the entire liver and ~1 gram of muscle tissue were taken. These samples were stored under liquid nitrogen until analysis for TNT and other metabolites was conducted. Also, all excreta was collected and held from each cage over the entire dosing period. At the termination of the experiment, the excreta from each group of animals were pooled and stored at -20°C.

5. Procedures for the Collection of Animal Tissues at the AAAP site

a. Sampling of animals at control sites.

Oak Ridge National Laboratory personnel were assigned to the hunting of animals at control sites (see Section III.4.a). Tissue samples (liver and muscle) obtained from deer, rabbit, and quail were transported on ice to the laboratory and stored under liquid nitrogen. A chain-of-custody for each sample was maintained.

b. Sampling of animals at AAAP site.

Consultants were hired to hunt animals at the AAAP site. Instruction for this hunt were in accordance with a detailed "Scope of Work." In essence, the consultants were asked to obtain tissue samples (muscle and liver) from deer, rabbit, and quail at the U.S. Army Alabama Ammunition Plant at Childersburg, Alabama. Samples of tissue were frozen (dry ice) as soon after collection as possible and a chain-of-custody for each sample was maintained. Collection of samples was observed by staff of the Agency for Toxic Substances and Disease Registry. All samples were returned to the Oak Ridge National Laboratory by ORNL courier.

6. Statistical Analysis

A statistical design is presented in Appendix A with the objective of deciding whether a chemical and its metabolites are likely to be present in the tissues of an animal population at or above a designated criterion level when only a portion of the population has been sampled. In the absence of such compounds in sampled animals at the detection level, the statistics are designed to quantify the confidence concerning absence of excessive contamination at the criterion level in the remaining population. In this regard, supplementary data analysis is provided in Appendix B to support the assumption that the magnitude of σ (standard distribution of a chemical in an exposed animal) can be described by a lognormal distribution. A Sampling and Analysis Decision Tree is presented that takes these constraints into account.

III. RESULTS

1. Extraction of Animal Tissues

Recovery data for deer liver tissue that was spiked with 1.25 ppm each of TNT-related compounds is tabulated in Table 1. The liver tissue was obtained from nonmunition-contaminated deer at the Catoosa Wildlife Management Area in East Tennessee (see Section III.4.a). The tissue was spiked in the laboratory with 1.25 ppm each of the compounds listed in Table 1 and extracted according to the standardized protocol (see Section II.3). The extract was analyzed by the mixed-mode HPLC method (see Section II.2). A discussion of the recovery data is found in Section IV.2.

Table 1. CORRECTED RECOVERIES OF TNT-RELATED COMPOUNDS FROM SPIKED DEER LIVER TISSUE.

Compound[a]	% Recovery
	± s.d. (n=3)
TNT (I)	84.0 ± 4.3
TNB (XI)	76.0 ±10.6
2,4-DA-6-NT (V)	53.3 ± 6.1
2,6-DA-4-NT (VIII)	41.0 ± 4.4
4A-2,6-DNT (IV)	111.6 ± 4.0
2A-4,6-DNT (VII)	113.3 ± 6.0
4HOA-2,6-DNT (II)	3.6 ±10.0
TNB alcohol (IX)	103.3 ± 7.8
TNB acid (X)	9.3 ±12.5
Azoxydimer (III)	4.3 ± 1.5
DNB (surrogate standard)	100[b]

[a] Roman numeral refers to designation noted in Figure 1.

[b] Absolute recovery was 56.7 ± 3.2%

2. Development of Analytical HPLC Procedures

An example of the separation of a standard mixture in acetonitrile containing 1 ppm (each) of TNT and metabolites by the mixed-mode HPLC method (see Section II.2 for details) is shown in Figure 2. The various chromatographic gradient parameters are summarized in Table 2. The total gradient program required 45 min to complete, including a final 7-min re-equilibration period at the end of the program.

Table 2. GRADIENT HPLC PROGRAM ON ANION/C18 COLUMN

Solvent A: 0.015 M Potassium Phosphate at pH 5.1 in 10:90 methanol:water.

Solvent B: Methanol

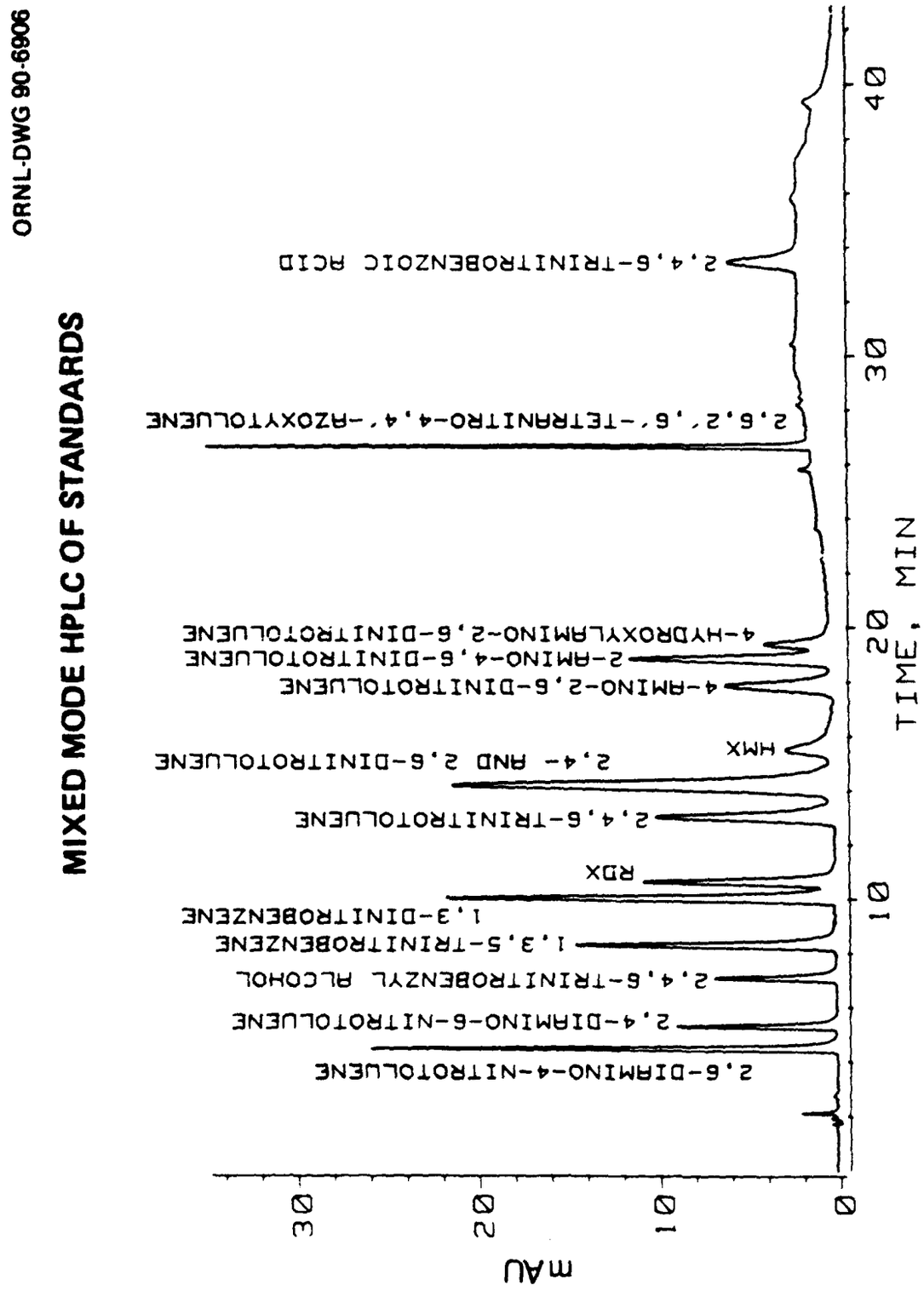
Solvent C: Acetonitrile

Time	% A	% B	% C
1	72	0	28
5	68	0	32
14	68	0	32
20	44	12	44
26	2	54	44
33	2	54	44
38	72	0	28
45	72	0	28

3. Biological Transformation of TNT

a. in vivo: Exposure of Mice to [^{14}C]TNT.

All mouse samples from the [^{14}C]TNT dosing experiment were analyzed for their [^{14}C] content. The procedure detailed in Section II.3 for the extraction of TNT-related compounds from animal tissue was applied to the muscle, liver and feces samples. At each step in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MIXED MODE HPLC OF STANDARDS
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10
20
30
TIME, MIN
10
20
30
40
2,6-DIAMINO-4-NITROTOLUENE
2,4-DIAMINO-6-NITROTOLUENE
2,4,6-TRINITROBENZYL ALCOHOL
1,3,5-TRINITROBENZENE
1,3-DINITROBENZENE
RDX
2,4,6-TRINITROTOLUENE
HMX
2,4- AND 2,6-DINITROTOLUENE
4-AMINO-2,6-DINITROTOLUENE
2-AMINO-4,6-DINITROTOLUENE
4-HYDROXYLAMINO-2,6-DINITROTOLUENE
2,6,2',6'-TETRANITRO-4,4'-AZOXYTOLUENE
2,4,6-TRINITROBENZOIC ACID

Figure 2.

the extraction procedure, an aliquot was analyzed for its [^{14}C] content. Urine and blood were extracted differently as no solid material was available for homogenization. Urine was extracted directly with $CHCl_3$ and blood was diluted with CH_3OH (1:3 vol:vol) and debris removed by centrifugation. All fractions obtained at the various extraction steps for each sample were counted and the recovery of radiolabel calculated. This data are reported in Table 3 and are discussed in more detail in Section IV.5.a.

An aliquot from the final fraction of each sample was taken for analysis by the mixed-mode HPLC method to determine whether the radiolabel present in that fraction co-chromatographed with authentic TNT-related compounds. However, the amount of radiolabel recovered in the "organic-phase" of the various extracted mouse samples, except for the urine, were not sufficient for a definitive analysis of free TNT-like compounds by the mixed-mode HPLC method.

TABLE 3. RECOVERY OF [^{14}C] IN MOUSE TISSUES AND EXCRETA[a]

	Muscle	Blood	Liver	Feces	Urine
Extraction Step:					
Homogenate	0.17±.08	-	2.75±1.20	16.77	-
Cell Debris	0.13±.11	-	0.71±.39	15.71	-
Aqueous Phase	0.02±0.3	0.15±.07	0.16±.20	2.95	128.00
Organic Phase	0.01±0.1	0.12±.07	0.16±.07	4.98	3.74

[a] Data normalized to micrograms of [^{14}C]TNT equivalents per gram or milliliter of sample ± s.d. (n=8). Excreta data represents average of duplicate determinations.

b. in vitro: Microsomal-mediated Degradation of TNT.

The liver tissue from deer that had not been environmentally exposed to TNT (see Section III.4.a[1]) was tested in vitro for its capacity to degrade TNT. An S_9 fraction (crude microsomal preparation) was prepared and incubated with TNT (Fu et al., 1983). After an incubation of 1 hour at 37°, the TNT and any metabolites formed were extracted from the

reaction mixture with ethylacetate and analyzed by the mixed-mode HPLC method (see Section II.2). The resulting chromatogram is detailed in Figure 3 for the deer liver and Figure 4 for a comparable commercially obtainable Aroclor 1254-induced rat liver S_9 preparation.

4. Collection and Analysis of Animal Tissues from Field Sites.

a. Field Collection Activities.

(1). Control Sites. Control samples of muscle and liver tissue from deer, rabbit and quail were obtained from nonmunition-contaminated animals. These animals were collected on the Oak Ridge National Laboratory reservation or at the Catoosa Wildlife Management Area in East Tennessee from January through August of 1989. The collection was under the supervision of personnel from the State of Tennessee Wildlife Resources Agency and all tissue collected were archived under liquid nitrogen at the Oak Ridge National Laboratory.

(2). AAAP Site. Animals were hunted on the Alabama Army Ammunition Plant site, Childersburg, AL from Sunday, November 26, 1989 through Tuesday, December 5, 1989. An accurate record of all samples was kept and a chain of custody maintained. All samples collected were archived at the Oak Ridge National Laboratory under liquid nitrogen and under lock and key. The inventory from this hunt include: liver and muscle tissue from 12 deer, 5 quail, and 5 rabbit. A complete critique of this portion of the project is provided in Appendix C.

b. Sample Analyses

A total of 168 separate analyses were performed on the various field-collected animal tissues to determine whether TNT-related chemicals were present. The number and type of samples processed are listed in Table 4. Each analysis consisted of the extraction of an individual tissue (or blank) sample followed by HPLC on a mixed-mode column and examination of the eluent for TNT and its metabolites. The results of each individual analysis was recorded on a separate "Summary Sheet" and examined by Dr. John Caton. A sample sheet of the data collected is found in Appendix D, as well as the BASIC program code developed for the detection limits.

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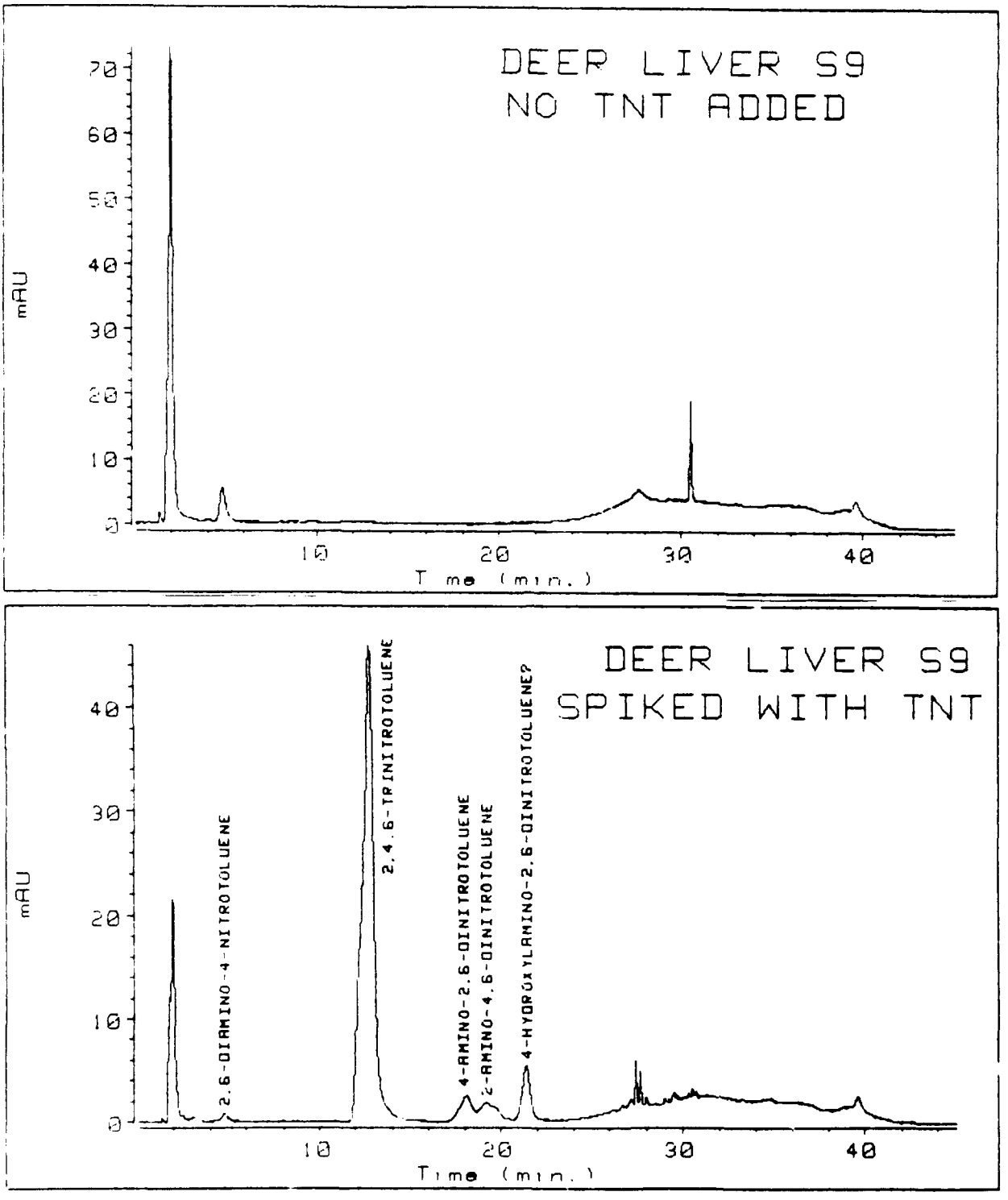

Figure 3.

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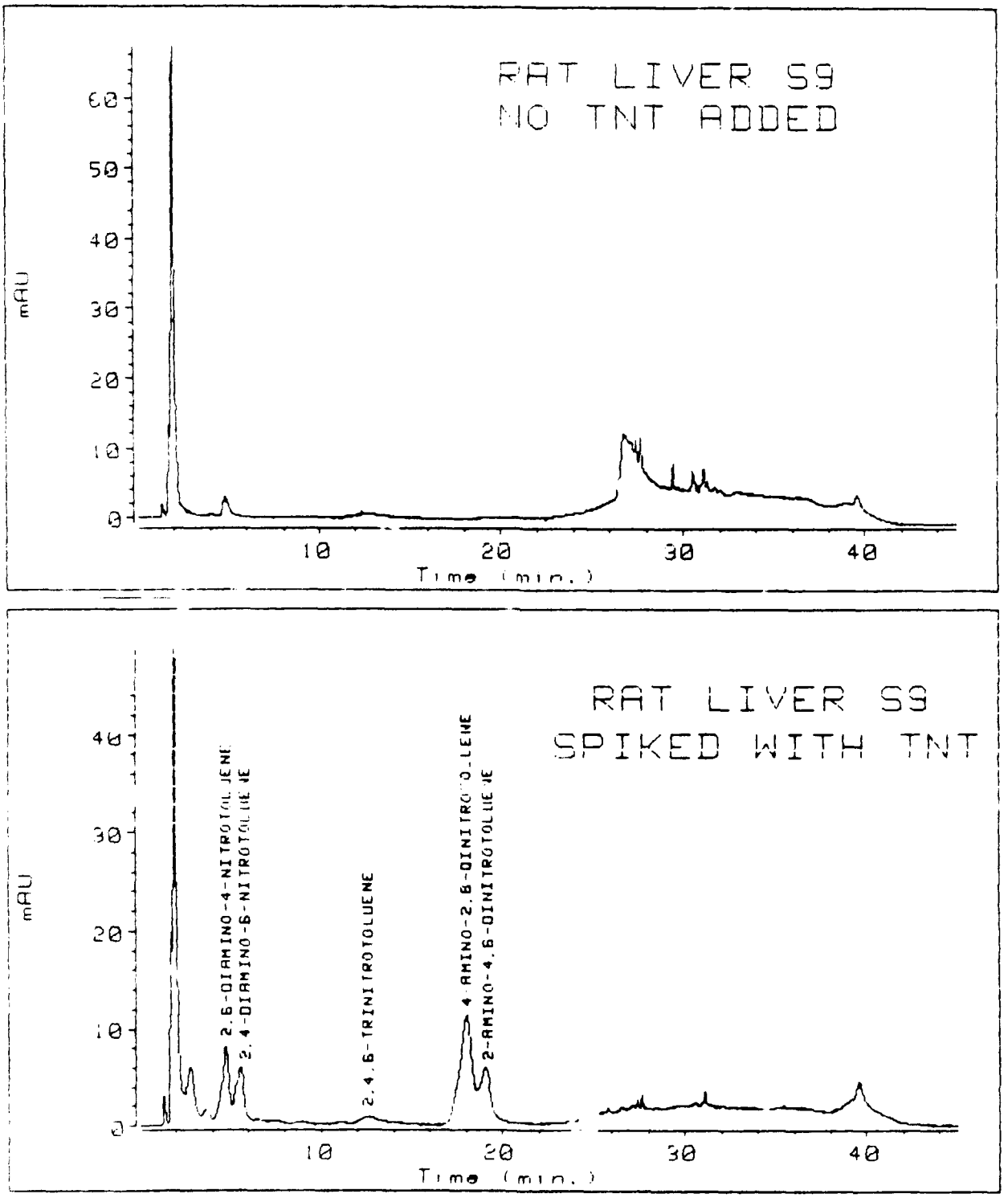

Figure 4.

TABLE 4. NUMBER AND TYPE OF FIELD-COLLECTED SAMPLES PROCESSED FOR TNT-RELATED CHEMICALS

Sample Type	Samples[a]	Controls[b]	Spikes[c]	Total
Deer Liver	36(12)	5	11	52
Deer Muscle	36(12)	3	3	42
Rabbit Liver	12 (4)	6	0	18
Rabbit Muscle	15 (5)	6	1	22
Quail Liver	7 (5)	2	0	9
Quail Muscle	15 (5)	5	2	22
Blank (no tissue)			3	3
Totals	121	27	20	168

[a] Samples indicate number of tissue preparations analyzed from the AAAP site. Number in parenthesis indicates the number of animals from which these samples were derived.

[b] Controls refer to processed tissue samples from animals taken from a non-contaminated site.

[c] Spikes refer to processed samples to which known amounts of several metabolites were added before processing.

IV. DISCUSSION/CONCLUSIONS

1. Scope of Work

The main objective of the work detailed in this report was to determine whether TNT and nine potential metabolites were present in the tissues of deer and other game animals on the Alabama Army Ammunition Plant site, Childersburg, AL at concentrations that would be unsuitable for human consumption. Because of the state-of-the-science for this type of project, it was necessary to accomplish several ancillary tasks in order to attain this objective. These tasks included the:

(i) Development of a protocol for the extraction of TNT-related chemicals from animal material.

(ii) Development of an HPLC method for the separation and detection of TNT-related chemicals.

(iii) Procurement/chemical synthesis of authentic chemicals for use as standards in the development of analytical methodologies.

(iv) TNT metabolism studies.

(v) Sampling of animals at the AAAP site.

(vi) Analysis of animal tissues taken from the AAAP site.

(vii) Design of a statistical strategy for the interpretation of results.

2. Extraction Procedure

An extraction procedures for the recovery of TNT and its metabolites from animal tissues was developed using liver and muscle from nonmunition-contaminated deer (see Section III.4.a). Tissue from these animals was spiked with TNT-compounds at or below the 1.2 ppm level (see Section I.1) and the recovery of added compounds was verified by appropriate HPLC. The extraction problem was approached by a combination of homogenization and solvent partitioning. The need for cellular disruption of tissue is well established and was met by a combination of blending and ultrasonic treatment. Although there is not an extensive literature of well-characterized analytical methods for the extraction of munitions-like compounds from biological materials, several reports provided useful guidance. Lakings and Gan (1980) showed good recoveries of RDX, DNT, and TNT spikes in muscle/fat samples which were homogenized

and extracted with acetonitrile. TNT recoveries ranged from 91% at 0.05 mg/kg to 84% at 0.2 mg/kg. Palazzo and Leggett (1986) reported 94-130% recoveries of 4 to 82 mg/kg of TNT, 2-A-4,6-DNT, and 4-A-2,6-DNT spiked in plant tissue and extracted overnight (extraction method not stated) in benzene. These experiences indicate that several solvents could be useful.

The following factors were considered paramount for the development of the extraction procedure adopted:

(i) Enrichment of those compounds of interest with a minimum co-extraction of extraneous matter.

(ii) Acceptable and reproducible recoveries of spiked authentic compounds.

(iii) Recovery of those compounds of interest in a matrix compatible with the analytical HPLC techniques used for detection and quantification.

The data in Table 1 indicate that overall the recovery and reproducibility was very good and that the extraction method performed well for the parent compound TNT, the monoaminodinitro metabolites, TNB alcohol, and TNB. Experiments showed that only the azoxydimer was sensitive to the nitrogen blow-down. The more polar diaminomononitro metabolites are extracted to a lesser extent, but recovery is satisfactory. TNB acid, 4HOA-2,6-DNT, and the azoxydimer are poorly recovered and the following specific problems dealing with chemical stability of these compounds were noted:

(i) TNB acid decomposes very quickly in acetonitrile. In fact, a freshly prepared solution of TNB acid in acetonitrile begins to show significant amounts of TNB and no TNB acid can be observed after 24 hours at ambient temperature. Decomposition begins immediately in 1:1 acetonitrile:water but proceeds more slowly. In aqueous solution TNB acid appears to be stable for an extended period of time. This observation may present some problems for the metabolite determination in tissue. In aqueous media such as tissue TNB acid would be stable; however, extraction of the metabolites from the tissue requires the use of an organic solvent in which any TBA would decarboxylate. Thus the analyzed sample may show TNB while the tissue initially contained some TNB acid.

(ii) The second stability problem concerns 4HOA-2,6-DNT which decomposes to the azoxydimer under the solvent conditions used for HPLC. This decomposition is observed immediately in a freshly prepared solution and proceeds slowly over an extended period of a week or more until the 4-HOA-2,6-DNT is no longer observed. Dependency of this instability on different solvent media has not been observed.

(iii) Finally, the azoxydimer demonstrates an instability attributable to photosensitivity.

3. HPLC Methodology

The separation of TNT, and several of its possible metabolites presented a challenging problem for a single liquid chromatographic determination. The problem was compounded by several constraints which were dictated by real samples (i.e., only a limited amount of sample can be made available from most animal tissue samples). Thus, it was quite desirable to analyze the sample in a single analytical determination thereby consuming a minimum amount of sample. This eliminated the utilization of most autosamplers which use copious amounts of sample to flush out and fill the injection system. However, the wide range of polarity of the compounds of interest generally indicated the need for an extended run time employing a wide solvent gradient. Such an extended run time made the use of an autosampler quite necessary in order to achieve a reasonable sample throughput rate. Not only will the amount of sample available be limited but also the concentration of the metabolites in the sample may be quite low. Therefore, the ideal method should be quite sensitive while utilizing the maximum sample volume.

The incorporation of both acetonitrile and methanol in building the gradient appeared to be necessary. Peak shape in the early portion of the chromatogram was much better when acetonitrile was the major portion of the organic phase of the eluting solution. However, if acetonitrile was used exclusively in the more rapidly changing portions of the gradient, (after 14 min), large momentary pressure fluctuations were observed. Some of these fluctuations were sufficient to terminate the operation of the more sensitive HPLC instrumentation systems. It is believed that these pressure fluctuations may result from a momentary precipitation of phosphate in a capillary or near a frit. At any rate, the substitution of methanol resolved this problem. However, for many HPLC systems where transfer lines are 0.25 mm or greater and frit porosity is 5 microns, it is quite probable that the gradient profile could utilize acetonitrile instead of

methanol. The separation on the mixed-mode column allows TNB acid to be determined along with the other anticipated metabolites. The HPLC procedure also is capable of detecting the explosives HMX and RDX if they are present above the Hubaux-Vos detection limits, but the instrument was not routinely calibrated for those explosives because they were not observed in the samples.

The detection limits for TNT and metabolites using the mixed-mode (anion/C18) HPLC method have been determined according to the procedure discussed by Hubaux and Vos, (1970), and are listed in Table 5. The BASIC program for this calculation is listed in Appendix D. Limits are presented for two different confidence levels. These limits represent the amount of compound that would be detected in animal tissue if two grams of tissue were extracted into a final volume of 0.5 mL with 50 μL of this extract then being analyzed. The data listed in Table 5 and the chromatogram shown in Figure 2 were generated from sample volumes of 50 μL, and thus they represent a realistic expectation for this method. More ideal results have been generated by injecting standards in volumes of 10 μL or less. Thus the method described yields very good results for most compounds even though a sample volume that is 5 or more times the ideal is injected.

In summary, method development focused on the determination of all compounds of interest in a single chromatographic run; the injection of a maximal volume of a dilute sample; and sensitivity.

Table 5. CHROMATOGRAPHIC PARAMETERS FOR EXPLOSIVES AND TNT METABOLITES ON MIXED MODE ANION/C18 COLUMN

COMPOUND	RET. TIME, MIN.	K[a]	SYM[b] MIN.	DET. LIMIT[c] 95, PPM	99, PPM
1,3,5-TRINITROBENZOIC ACID	34.0	17.3	1.3	0.07	0.10
2,4-DIAMINO-6-NITROTOLUENE	5.7	2.1	1.1	0.09	0.12
2,6-DIAMINO-4-NITROTOLUENE	4.8	1.6	1.3	0.03	0.04
TRINITROBENZYL ALCOHOL	7.2	3.3	0.84	0.13	0.18
RDX	11.0	4.9	0.72	0.10	0.14
HMX	14.8	7.0	1.2	0.09	0.14
1,3-DINITROBENZENE	10.1	4.5	1.2	0.05	0.07
1,3,5-TRINITROBENZENE	8.3	3.5	1.2	0.05	0.07
2-AMINO-4,6-DINITROTOLUENE	19.1	9.3	1.1	0.15	0.22
4-AMINO-2,6-DINITROTOLUENE	18.2	8.8	0.93	0.06	0.09
2,6-DINITROTOLUENE	14.4	6.8	1.1	0.15	0.20
2,4-DINITROTOLUENE	14.5	6.8	1.1	0.12	0.16
2,4,6-TRINITROTOLUENE	12.8	5.9	1.1	0.05	0.10
4-HYDROXYAMINO-2,6-DINITROTOLUENE	20.2	9.9	1.2	0.2	0.34[d]
2,6,2',6'-TETRANITRO-4,4'-AZOXYTOLUENE	26.6	13.3	1.3	0.5	0.7

[a] The K-value, (capacity ratio), is the corrected retention time divided by the time required for an unretained compound to travel through the system.

[b] SYM is the total peak area after the apex divided by the total peak area before the apex.

[c] DET. LIMIT is the detection limit in PPM for tissue extracts assuming two grams are extracted into a final volume of 0.5 mL with 50 μL of this extract injected. These detection limits have been calculated for the 95 % and 99 % confidence levels according the methods suggested by Hubaux and Vos (1970).

[d] The detection limits listed for 4-HOA-2,6-DNT must be considered approximations because this compound has limited stability under the chromatographic conditions employed.

4. Authentic Standards

All authentic standards needed for the work detailed in this report were obtained except for one compound. Attempts, as well as those of Sitzmann (1974), to prepare 2-hydroxyamino-4,6-dinitrotoluene (2HOA-4,6-DNT) by the method of Nielsen et al. (1979) or by the method of McGookin et al. (1940) were unsuccessful. Further literature surveys questioned whether this compound had ever been truly synthesized (see Sitzmann, 1974). We noted some confusion in the literature between the 2- and 4- isomers. Nielson et al (1979) was only able to report a speculated NMR spectrum of 2HOA-4,6-DNT in the 4HOA-2,6-DNT reaction mixture. All of these observations suggest that 2HOA-4,6-DNT has never been synthesized.

5. Metabolism Studies

a. in vivo Studies with [^{14}C]TNT.

From the recovery data reported in Table 3, the distribution and concentration of [^{14}C] in various tissues and excreta of a 30 g mouse upon termination of the [^{14}C]-TNT dosing experiment were calculated and are given in Table 6. These data show that during the nine-day dosing study, a small but detectable amount of radiolabel accumulated in tissues of the mice examined. Of the total dose administered (3 mg of [^{14}C]TNT per mouse), only 5.92 μg (0.2 %) was recovered in the blood, liver and muscle tissues. Another 516 μg (17.2 %) was found in the excreta. As only >20% of the total dose administered can be accounted for in these parts of the animal, it is assumed that the remaining radiolabeled material is still associated with the carcass, probably in the intestinal tract.

That portion of free TNT and metabolites present in the [^{14}C] reported in the muscle and liver tissue (Table 6) can be inferred from the amount of the radiolabel that partitions into the organic phase during the extraction of these tissues (Table 3). This is calculated to be ~11 % of the total amount found for either tissue (i.e., the percent found in the organic phase relative to the homogenate). It should be noted that this is a corrected value based on the anticipated recovery noted with spiked TNT and metabolites from animal tissue (~50%, see Table 1). Thus the estimated concentration of free TNT and its metabolites that accumulated during the controlled chronic exposure of an experimental animal was 20 and 300 μg/kg of muscle and liver tissue, respectively. These tissue concentrations of free compounds are below the study design criteria levels established for game-animal tissues. However, since a significant amount

Table 6. DISTRIBUTION AND CONCENTRATION OF [^{14}C] IN MOUSE TISSUES AND EXCRETA AT TERMINATION OF DOSING

Distribution[a]	Concentration[b]	
	μg	%
Tissue		
Blood (2 mL)	0.3	0.01
Muscle (12 g)	2.04	0.07
Liver (1.3 g)	3.58	0.12
Excreta		
Feces (1.25 g)	20.96	0.70
Urine (3.9 mL)	496.00	16.53

[a] Number in parenthesis refers to estimated amount of blood and muscle for a 30 g mouse or average amount of liver tissue or excreta actually collected per mouse.

[b] Numbers calculated using recovery data from Table 3. Concentration reported as total amount in micrograms present in stated amount or tissue or excreta; or as the percent of total dose administered.

of the radiolabel in the muscle and liver was non-extractable, one must conclude that perhaps additional metabolism of TNT occurred and other products accumulated. Based on our current knowledge of cellular detoxication and metabolism of xenobiotic chemicals it would not be unreasonable to expect that TNT metabolism would result in the adductions of macromolecules such as proteins and nucleic acids (radiolabel associated with cell debris), and the formation of conjugated intermediates (radiolabel that does not extract into organic phase), thus accounting for this additional 89% of non-extractable [^{14}C] found in muscle and liver. It should be noted that the analytical procedures developed for this study were not designed to identify these "other"

forms of [^{14}C] radiolabel. Although it is not known whether these products would constitute a human health problem if present in tissues of game animals exposed to TNT, it is known that adduction of macromolecules, specifically DNA, has been implicated in chemical carcinogenesis (Williams, 1987), while conjugation reactions are known to play an important role in the formation of toxic metabolites (Monks, et al., 1990). Therefore it would seem prudent to investigate this problem further.

Figure 5 details the distribution of [^{14}C] activity in the chromatogram generated by mixed-mode HPLC analysis of the urine both before and after extraction with chloroform. In unextracted urine (aqueous fraction), there is a major peak of [^{14}C] activity that elutes at the breakthrough of the run and a smear of activity across the remaining chromatogram. However, two distinct peaks of [^{14}C] activity are found in the chloroform extract; the major peak which represent ~70% of the total activity co-chromatographs with TNB alcohol while the minor peak does not co-chromatograph with any of the authentic metabolites available for this study. Although excreta, in particular the urine, contain a considerable amount of the administered dose, they were collected and pooled over the exposure period (9 days) in a non-sterile condition. Therefore, potential degradation of TNT and excreted products contained therein could have occurred due to the presence of microbial contamination, thereby complicating interpretation of products found.

b. in vitro Studies.

This portion of the study was designed to provide additional information concerning the capacity of liver tissue of both the deer and rodent to metabolize TNT. It was found that liver microsomes prepared from both rat and deer tissue mediated the degradation of TNT and produce qualitatively similar products. The data in Figures 3 and 4 indicate metabolism of TNT occurred via the hydoxylamino-intermediate to the diamino-type derivatives of TNT (see Figure 1.). As anticipated, the uninduced deer liver does not degrade TNT as completely, for a given incubation period, as the induced rat liver preparation (note presence of TNT in the chromatogram of Figure 3.). However, detectable product formation from TNT by deer not previously exposed to this compound suggest that these animals have an endogenous capacity to metabolize TNT. Furthermore no unexpected metabolism occurred as no new or unidentifiable UV-peaks were noted in these preparations. These data are important in that they demonstrate the feasibility of extrapolating results on TNT exposure across species. Another major implication

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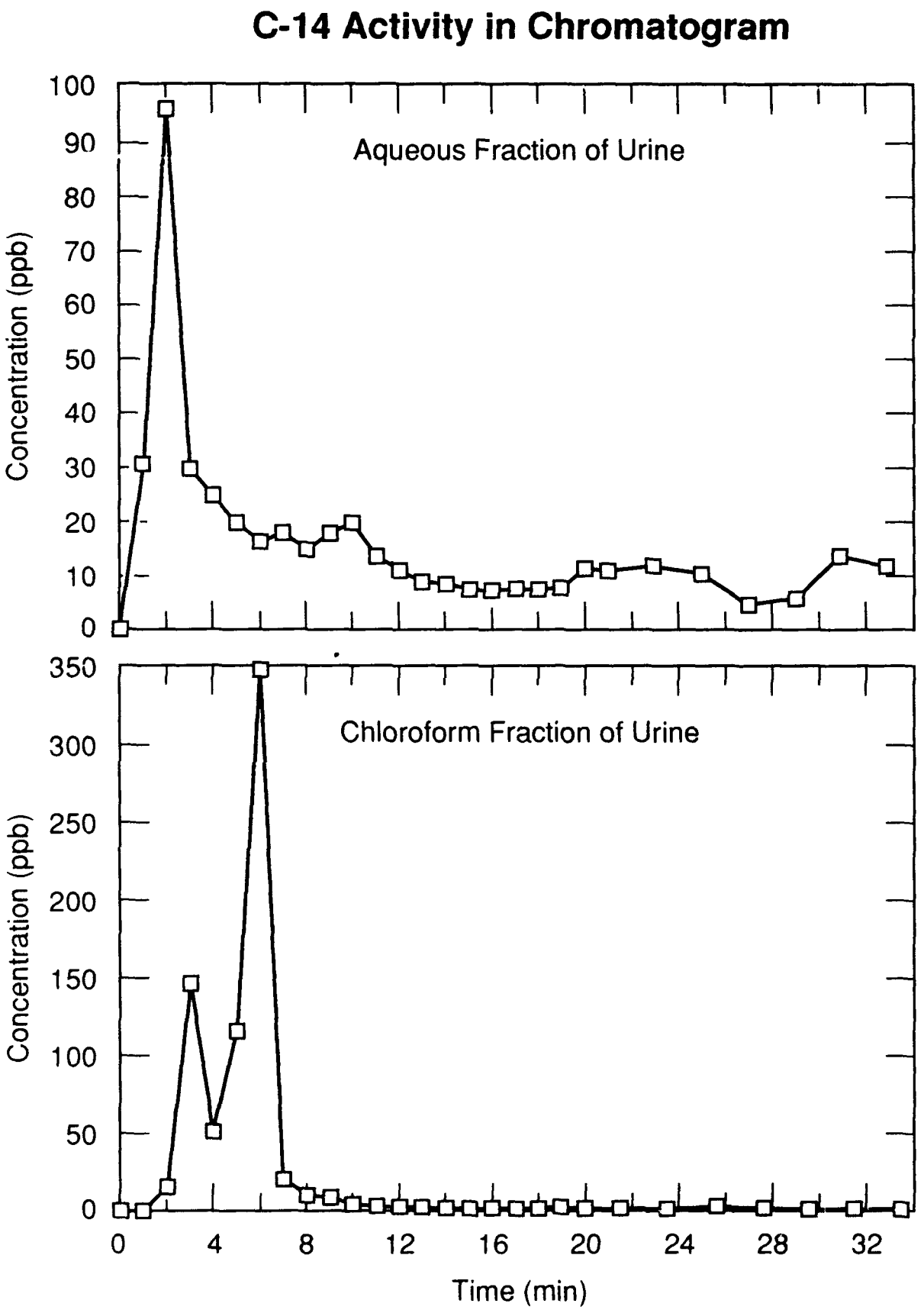

Figure 5.

of these studies is that TNT is probably rapidly metabolized and excreted or bound. Very little remains in the non-transformed or free state within the animal. Thus, it is not surprising that the analysis of animals harvested at AAAP (see Section IV.6) did not show any significant accumulation of free TNT or its metabolites.

6. Determination of TNT and Metabolites in Animals from AAAP Site

a. Sampling of Field Animals.

All activities concerning the hunting of animals for this study were under the supervision of authorized State Officials and trained professional hunters.

Animals from the control sites were readily available and several kilograms of various tissues from different species were archived for analysis.

Animals were not readily available from the AAAP site and the number of each species caught was below that which was anticipated (see Appendix C). Initial comments provided by the "on-site" personnel in charge were that:

(i) The deer population was lower than estimated (probably nearer 300 animals).

(ii) Numerous predators were observed, including a large feral dog population and, occasionally, humans.

(iii) During the time of the hunt, weather conditions were unfavorable.

Nevertheless, sufficient tissue was obtained from a total of 168 separate analyses (Table 4).

b. Analysis of Animal Tissues.

Of the 168 separate analyses on field-collected animal samples, one was completely lost during filtration.

The recovery standard was 1,3-dinitrobenzene, (DNB), which was added to each tissue at a level of 1.25 PPM. Recoveries generally ranged from 40% to >60%. Because DNB is more volatile than many of the target analytes, its recovery was often lower than the most relevant analytes, (see results from spike recoveries); thus, for example, one would generally expect the recovery of TNT to be higher than the recovery of DNB. The level of recovery for each sample is recorded on the results sheet for that sample. One might rate the reliability of results in terms of recoveries as follows:

% Recovery of DNB	Reliability of results
>50%	Very Good
40-50%	Good
30-40%	Fair
20-30%	Marginal
10-20%	Poor
<10%	Unacceptable

With this criterion the great majority of results fall in the good or very good range: 85% (23 out of 27) of the control samples analyzed and 78% (93 out of 120) of AAAP samples analyzed.

The recovery of different analytes varies. Compounds with greater water solubility such as 2,4-diamino-6-nitrotoluene and 2,6-diamino-4-nitrotoluene tend to have lower recoveries from the sample preparation process employed. Also, the tissue extraction process tended to generate an overall background which interfered with the analysis at the 0.1 PPM levels. Thus, the low-recovery spiked samples generated from control deer liver, (0.125 PPM), tended to show recoveries in the range of 100%-200% indicating a background contribution from the tissue on the order of 0.1 PPM. In contrast, the control samples spiked at the normal 1.25 PPM level generally showed recoveries in the range of 50%-80%. [At the PPM level the background is small compared to the spiked analyte level.] For some samples the background interferences generated from the tissue preparation appeared to be large. Major interferences most often tended to be in the chromatographic region where 2,4-diamino-6-nitrotoluene, 2,6-diamino-4-nitrotoluene, and 2,4,6-trinitrobenzyl alcohol eluted.

Although the determined detection limits ranged as low as 0.05 PPM for some analytes, the background contributed by the tissue preparation process tends to raise the practical detection limit to levels on the order of 0.1 PPM. Thus for practical purposes one should regard any result below 0.2 PPM with some question. With such criterion one would conclude that none of the target analytes were present in the samples at levels greater than 0.2 PPM or their stated detection limit, (whichever is higher). Without further investigation one can not rule out the possibility that some target analytes may be present at lower levels. Reanalysis using a confirmation column with a different separating mechanism, (e.g., normal phase vs reversed

phase), might reduce the background/interference contribution. At any rate the prepared samples have been stored in the dark at 4°.

c. Statistical Analysis and Significance of Data.

The purpose of the "Statistical Design" presented in Appendix A is to provide an approach for analyzing data on body burdens of TNT, the parent compound, and its metabolites in relation to a criteria level. In the absence of such compounds in excess of a design study criteria level for detection, the statistics are designed to quantify the confidence that excessive contamination in the population of animals would be detected. That is, one would be able to estimate the proportion of the total population of a species that contained body burdens above the criterion level so that some regulatory action may be taken.

Two sampling scenarios with their own set of statistical considerations were prepared. The first scenario is based on obtaining body sufficient burden data (i.e., in the sense of being able to characterize the distribution) above the detection limit of the analytical procedure. The second scenario is based on obtaining no body burden data (i.e., all analyses below the detection limit of the analytical procedure).

In the results reported in this study, none of the tissues analyzed was found to contain TNT or its metabolites above the detection limit of 0.2 PPM, therefore a statistical strategy that follows the rationale detailed for scenario 2 was applied to the data.

The following exercise was performed with the data obtained from AAAP site deer tissue (refer to Appendix A for details):

For a deer population size of N = 300; a random sample size of n = 12; and with zero observed number of animals with an individual metabolite concentrations greater than the detection limit (DL) of the analytical procedure used; then from Wright's Tables, we find the upper 95% confidence limit on the proportion of the population that exceeds the DL to be 0.22 (i.e., we are 95% confident that no more than 22% (65/300) of the deer population exceeds the DL for an individual compound).

This confidence statement about the proportion of the population that exceeds the DL can be converted to a confidence statement about the proportion greater than the CL if we assume the distribution of TNT-related observations are lognormal (see Appendix B) and that ln CL is $k^*\sigma$ units above ln DL. The upper limit on the proportion > DL may be converted to an upper limit on the proportion > CL, by finding the proportion of the distribution > $\mu+(0.78+k^*)\sigma$, where the value k^* is defined as $((\ln CL - \ln DL)/\sigma)$.

For σ = 0.887 (the value for σ is taken from Appendix B), and CL and DL in PPM, k^* = (ln 1.2 - ln 0.2)/.887 = 1.792/.887 = 2.02
Then from standard statistical tables on the proportion of the normal curve (one-tailed) that lies beyond a given normal deviate, we can say with 95% confidence that this proportion is 0.0026. Thus, no more than 0.26% of the 300 deer exceed the CL limit, or approximately 1 deer.

An important consideration in the above exercise is the assumption concerning the magnitude of σ and that the distribution of observation of TNT-related compounds is lognormal. In this regard, these consideration were evaluated from a statistical analysis of data obtained on the high-resolution gamma-ray analyses for ^{137}Cs content in the muscle and liver tissue of road-killed deer samples at the Oak Ridge National Laboratory during the 1983 calendar year. Deer are known to come in contact with ^{137}Cs as a result of their normal drinking and browsing activities on the ORNL Reservation, similar to the type of contact anticipated for munitions-like chemicals at the AAAP site. A summary of the statistical analysis for the road-killed data and the estimation of σ, is given in Appendix B.

The significance of the body burden data for TNT-related compounds in rabbit and quail from the AAAP site is uncertain since a statistical analysis could not be performed as with the deer data. This was because neither an accurate population estimate of these animals at the AAAP site was available nor a documented σ value, calculated from an exposure data base that was lognormally distributed. Nevertheless, it is encouraging that none of the tissues analyzed from these animals contained TNT-related compounds above the detection limit of the method of analysis and is probably indicative of negligible environmental exposure by these species to these compounds.

The "Analysis Decision Tree" from Appendix A can be appropriately applied to the data obtained on the animals from the AAAP site. In the case of the deer, it appears that the upper limit on P^* is acceptable, that is, there is a 95% confidence that no more than 0.26% of the deer population contain an individual TNT-related compounds in their tissues at or above the stated criteria level of 1.2 PPM. Therefore, one would conclude that no health danger exists. With the other species (quail and rabbit) an insufficient sample size was obtained and further work is necessary before the health issue with these animals can be documented.

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(1) Bibliography of Publications and Meeting Abstracts:
None to date.

(2) Personnel receiving pay from this contract:

Oak Ridge National Laboratory (personnel who worked on the project)

L. R. Shugart
J. F. McCarthy
B. D. Jimenez
E. Tan
F. V. Sloop, Jr.
C. Guzman
C. Gettys-Hull
K. Wilkinson
W. H. Griest
C. -H. Ho
B. A. Tompkins
J. Caton

Sub-contract (hunters)

Wayne E. Downing
Morgan A. Downing
Dean Downing

APPENDIX A

STATISTICAL DESIGN: STRATEGY FOR SAMPLING AND ANALYSIS

DEFINITIONS

N population size

n number of animals analyzed (SAMPLE)

A number of animals in population with levels of TNT metabolites exceeding CL

a number of animals in sample with levels of TNT metabolites exceeding cL

P^* proportion of population > critical level of TNT = A/N

CL critical level of TNT metabolites in animals

DL detection limit for quantifying TNT metabolites

β proportion of distribution covered by tolerance interval

γ confidence level

μ and σ

pertains to the unknown mean and standard deviation, respectively, for the ln metabolite distribution

$\bar{y}$ and v

pertains to sample estimate of μ and σ, respectively

INTRODUCTION

The objective of the sampling wild animals at the Alabama Ammunition Plant is to estimate P^*, the proportion of the total population of a species on the site that contains body burdens of TNT and metabolites above a "critical level" (CL) >1.2 mg/kg. A statement about P^* derived from a statistical analysis of the data collected should have a stated degree of confidence based in part on the detection limit (DL) of the analytical techniques employed. Therefore, two sampling scenarios are described that allow for statistical analyses to be performed on the data in relation to the DL. A Sampling and Analysis Decision Tree (Exhibit 1) is presented that takes these constraints into account.

SAMPLING SCENARIOS AND STATISTICAL CONSIDERATIONS

Scenario #1.

If the tissue levels of TNT and metabolites are sufficiently high relative to the detection limit (DL) of the analytical technique employed, then the distribution of the metabolite concentrations can be characterized by a normal or lognormal distribution. P^* can be estimated from the observed sample proportion > CL, i.e., by a/n. Tolerance limits can then be constructed on the proportion of the population > CL, and a statement can be made that we are $100\gamma\%$ confident that the proportion of the population > CL is no more than $1-\beta$ (Guttman, 1970). The following is an example of "Tolerance Intervals and Sample Size for TNT Metabolite Study".

If the normality assumption is appropriate for the observed or transformed (e.g., log) metabolite concentrations, then $\bar{y}$ and v are the mean and standard deviation, respectively, calculated from the observed or transformed observations. The one-sided β-content tolerance interval is of the form $(-\infty, \bar{y} + k'v)$ and, for the appropriate value of k', a statement of the following form can be made:

We are $100\gamma\%$ confident that at least $100\beta\%$ of the distribution of y is less than $\bar{y} + k'v$.

If CL is a critical or action level for y, then once we have a random sample we may solve

$$\bar{y} + k'v = CL$$

for

$$k' = \frac{CL - \bar{y}}{v} .$$

From this value of k', for a fixed value of γ we can determine the proportion of the population that is greater than CL.

Example: If CL = 4, $\bar{y}$ = 2, and v = 0.75 from a sample of size n = 10, then

$$k' = \frac{4-2}{0.75} = 2.67$$

If γ = 0.95, then from Table 4.6 [Guttman (1970), page 90], we find $\beta > 0.90$. However, if γ = 0.90, then $\beta > 0.95$. In the first case, we could say that we are 95% confident that no more than 10% [100(1-β)] of the distribution is greater than CL. In the second case, we could say we are 90% confident that no more than 5% [100(1-β)] of the distribution is greater than CL.

In order to estimate the sample size required to draw conclusions about β with acceptable statistical confidence, it is necessary to determine a range on $\bar{y}$ and v from which a range on k' can be obtained. From the minimum value of this range on k', we can find, for a fixed value of γ, the sample size needed to have β greater than a specified value.

Example: If we assume, or have reason to believe, that the ranges of $\bar{y}$ and v will yield a minimum anticipated value of k' to be 3.0, and if γ = 0.95, then for

(i) $\beta > 0.95$ we must have $n \geq 10$.

(ii) $\beta > 0.99$ we must have $n \geq 35$.

In addition, we could assume that the CL is k' standard deviation units greater than $\bar{y}$. For given values of γ and β with this value of k', we could then use Table 4.6 from Guttman (1970) to determine the sample size associated with these values of γ, β, and k'. Table 1 gives the values of n for different combinations of γ, β, and k'.

Example: Assume that we anticipated the CL to be not more than 3 standard deviation units above $\bar{y}$. From Table 1, a sample on 6-7 animals would be required if we want to be 90% confident (γ = 0.90) that no more than 5% (1-β = 0.05) of the distribution of metabolite concentrations (in log-units) will be above $\bar{y}$ + 3 v.

Scenario #2.

It may not be possible to estimate μ and σ because the concentrations of TNT and metabolites in the tissues of the animals are below the DL of the analytical technique employed. In that case, the objective of the sampling is to demonstrate that, with some statistical certainty, no more than x% of the population exceeds CL (in this discussion a requirement that $P^* \leq 5\%$ is assumed). We can approach this problem based on the tables of Wright (1991) for sampling of finite populations (see Table 2). Furthermore, we will assume that the body burdens within the population are distributed lognormally.

For example, let us assume a population size of N = 400 and a random sample of size n = 10, then if a, the observed number of animals with metabolite concentrations greater than the DL, is zero, we may use Wright's tables (with a = 0) to find the upper 95% confidence interval on the proportion of the population that exceeds the DL to be 0.26 (i.e., we are 95% confident that no more than 26% (102/400) of the population exceeds DL). Furthermore, by assuming knowledge about the difference between DL and CL we can convert the confidence statements about the proportion greater than DL to statements about the proportion of the population greater than CL. For a lognormal distribution, $Y = \ln X$ is assumed to have a normal distribution with mean μ and variance σ^2 and the point that cuts off 0.26 of the upper portion of this distribution (obtained from data on the Area of the Normal Curve) is given by $(\mu+0.64\sigma)$. If the additional assumption is made that ln CL is $k^*\sigma$ units above ln DL, then the upper limit on the proportion > DL may be converted to an upper limit on the proportion > CL, by finding the proportion of the distribution > $\mu+(0.64+k^*)\sigma$. The value k^* is defined as $((\ln CL - \ln DL)/\sigma)$.

This formulation demonstrates that the statistical certainty of any statements about estimates of P^* will depend on the following variables (Table 2):

1. The number of animals sampled (n) and the number exceeding the DL or CL;
2. The magnitude of the difference between CL and DL, expressed in log units; and
3. Assumptions about the magnitude of σ.

For example, if we assume that the variance is one log unit (σ = 1) and the CL is one log unit greater than DL, then $k^* = 1$. If the DL is lowered an additional log unit, then $k^* = 2$. As an additional example, assume that we sample 10 animals and find none with TNT metabolite levels above the DL. If $k^* = 1$ (i.e., CL is one log unit > DL), then we can state with 95% certainty that P^* is not more than 5%. If we decrease the DL by one log unit, k^* becomes 2.0, and there still were no animals above the DL, we are assured that no more than 0.4% of the population is above CL. If however, the lower DL enables us to detect measurable levels of metabolites in one animal (Table 2, a=1, n=10, k^*=2.0), then no more than 1% of the population exceeds the CL. In this formulation, the ability to make statements about P^* depends on the number of animals sampled and the difference between CL and DL.

This analysis is also sensitive to assumptions about the magnitude of σ, which cannot be experimentally determined if only a small proportion of the population exceeds DL. Therefore prior knowledge about the magnitude is required.

In the design of this sampling strategy, two key variables can be controlled:

1. Number of animals collected. For large animals such as deer, there is, realistically, an upper limit on the "reasonable" number of animals that can be sampled. The number of deer that need to be sampled to provide acceptable tolerance limits on P^* cannot be determined a priori, but preliminary estimate suggest that approximately 10-20 animals may be sufficient (Table 3), and this is a number that seems reasonable from a logistical perspective. However, much larger numbers of small animals, such as rabbits and quail, can be sampled.
2. The DL of the analysis for TNT metabolites can be improved by increasing the volume of tissue extracted. For large animals, the DL could be improved by scaling up the extraction procedure to work with more tissue and this would decrease the DL. The smaller size of the rabbits and quail limit the extent to which the DL could be improved for these species.

SAMPLING STRATEGIES

Based on the considerations discussed above, we propose the following strategy (Exhibit 1):

1. Collect 10-20 deer (see discussion under 3.1.3, below), all of the same age and sex (comparison of sexes would require another 10-20 animals). Archive (freeze) samples of muscle and liver in the quantities of 10g and 100g (each in triplicate).

2. Collect at least 20 rabbits and quail of the same age and sex. Freeze triplicate quantities of 10g samples.

3. Extract and analyze TNT metabolites from 10 individuals for each species/tissue and evaluate the results:

3.1. If most samples > DL (sampling scenario #1), then estimate μ and σ^2 for each population and tissue. Estimate proportion of the population greater than CL and estimate tolerance limit on population:

3.1.1. If the portion of the population > CL is acceptable (e.g., $P^* < 5\%$ of the population), and the tolerance limits are acceptable, then conclude that no health danger exists and end the study.

3.1.2. If the portion of the population > CL is unacceptable and the tolerance limit acceptable (e.g high confidence that $P^* > 5\%$ of the population) accept that the animals present a potential danger to hunters and end the study.

3.1.3 The confidence in an estimate of the P^* is low (e.g., $\gamma < 0.9$), then the statistical confidence of the estimate can be improved only by increasing the sample size. If additional animal samples are available, these can be analyzed to increase the confidence of the estimate of P^*. It must be recognized that a cost/benefit decision must be made prior to sampling: a balance must be achieved between the effort involved in collecting (and potentially not needing) additional animals, compared to the benefits of being assured that the final analyses will permit statistically acceptable statements about P^*.

3.2. If most samples < DL (sampling scenario #2), but the upper limit on P^* is acceptable (i.e., there is greater than 95% confidence that $P^* \leq 5\%$ of the population, given a scientifically defensive assumption on the value of σ and based on an analysis as illustrated in Tables 2), then conclude that no health danger exists and end the study.

3.3. If most samples < DL (sampling scenario #2), but the upper limit on P^* is unacceptable (i.e., it cannot be stated with 95% confidence that $P^* \leq 5\%$ of the population), then two choices exist: accept that the animals may present a potential danger to hunters and end the study, or improve confidence in the conclusion by gathering more data to improve statistical power.

3.3.1. If more data need to be analyzed, different strategies are employed for different species (Table 2):

- For small animals, the mass of tissue available for analysis is limited, but more animals are available for analysis. Therefore, the statistical power will be increased by analyzing some or all of the animals held in reserve.
- For deer, sample volume is not limited, so we will decrease DL by extracting the larger volume tissue samples held in reserve. Animals held in reserve can also be analyzed to increase sample number, but inspection of Table 2 suggests that a decrease in DL (which increases k*) will have a greater effect in improving statistical confidence.

REFERENCES

Guttman, I. (1970). Statistical Tolerance Regions: Classical and Bayesian. Charles Griffin and Co., Ltd., London.

Wright, T. (1991). Exact Confidence Bounds When Sampling From Small Finite Universe-An Easy Referernce Based on the Hypergeometric Distribution. In: Lecture Notes in Statistics, Springer-Verlag, New York, in press.

Table 1. Sample Sizes for Combinations of γ, β, and k'

(a) $\gamma = 0.90$

k'	$\beta = 0.90$ $1-\beta = 0.10$	$\beta = 0.95$ $1-\beta = 0.05$	$\beta = 0.99$ $1-\beta = 0.01$
1.5	76-77	>1000	>1000
2	11-12	42	>1000
2.5	5-6	11-12	230-240
3	4-5	6-7	22-23
3.5	3-4	4-5	10-11
4	3-4	3-4	6-7

(b) $\gamma = 0.95$

k'	$\beta = 0.90$ $1-\beta = 0.10$	$\beta = 0.95$ $1-\beta = 0.05$	$\beta = 0.99$ $1-\beta = 0.01$
1.5	120-130	>1000	>1000
2	17-18	66-67	>1000
2.5	8-9	16-17	375
3	6	9-10	34-35
3.5	4-5	6-7	15-16
4	4	5-6	9-10

(c) $\gamma = 0.99$

k'	$\beta = 0.90$ $1-\beta = 0.10$	$\beta = 0.95$ $1-\beta = 0.05$	$\beta = 0.99$ $1-\beta = 0.01$
1.5	240-250	>1000	>1000
2	31-32	120-130	>1000
2.5	15-16	30-31	700-800
3	10-11	16-17	65-66
3.5	7-8	11-12	28-29
4	6-7	8-9	17-18

Table 2. **Upper 95% Confidence Limit On P^***
For a Population at Size N = 400[1]

n	a	k^*		
		0.5	1.0	2.0
10	0	0.13	0.05	0.004
	1	0.22	0.10	0.01
	5	0.61	0.41	0.11
20	0	0.06	0.02	0.001
	2	0.14	0.06	0.005
	10	0.50	0.31	0.07
30	0	0.03	0.01	0.0004
	3	0.11	0.04	0.003
	15	0.46	0.28	0.06

[1] Derived from information in Wright (1991).

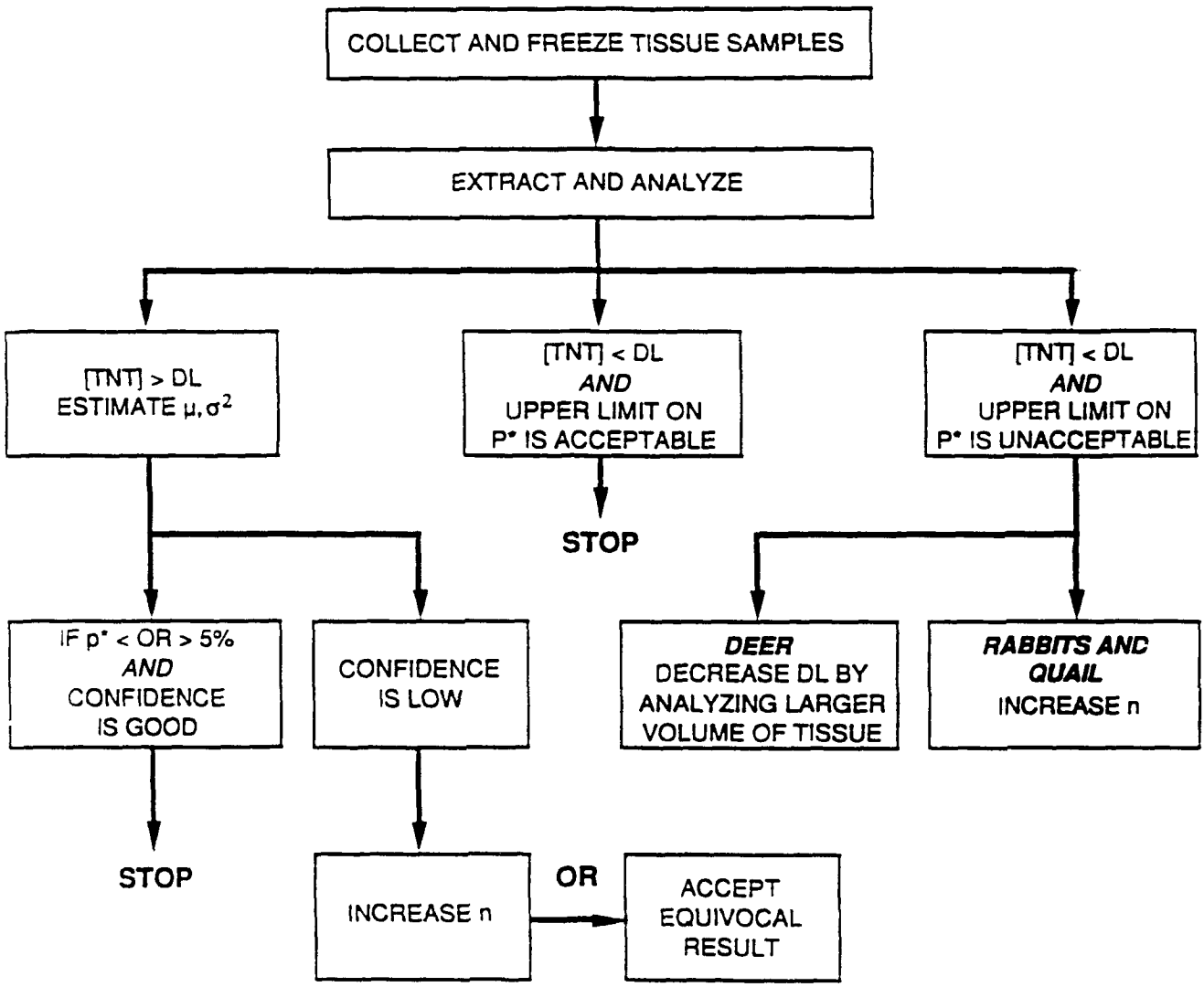
Appendix A

Exhibit 1

SAMPLING AND ANALYSIS DECISION TREE

ORNL-DWG 89M-13837

SAMPLING AND ANALYSIS DECISION TREE



APPENDIX B

ANALYSIS OF 1983 ROAD-KILLED DEER DATA

The purpose of this data analysis (using the ^{137}Cs data in muscle and liver only) was to determine if these concentrations could be adequately described by a lognormal distribution. There were 81 observations for the muscle data with 3 of these being less than detection limit (not fixed). There were 74 observations for the liver data with 25 of these being less than the detection limit (i.e., more than a third of the liver data was less than the detection limit).

In order to test for the adequacy of the lognormal assumption, the observed concentrations were transformed (log) and these transformed values were tested against a normal distribution. Two methods were used to handle the observations "less than" the detection limit:

Method 1. Replace the "less than" values by one-half the detection limit. As long as the number of observations in question is a small percentage of the data set, this is a reasonable approach.

Method 2. Remove all "less than" values from the data set. This has the disadvantage of producing biased estimates of the mean and variance of the distribution under consideration.

Table 1 is a summary of the test for lognormality using the SAS UNIVARIATE Procedure. For the muscle data, both methods indicate the reasonableness of the lognormal distribution and also the small effect the 3 "less than" observations had on the results. However, for the liver data, the two methods produce somewhat different results. This is caused primarily because of the large number of censored observations. The significance of the liver results for Method 1 is probably caused by the large number of observations with identical values (e.g., 6 observations with <20 values are replaced by 20/2 = 10).

Table 1. GOODNESS OF FIT TEST

Tissue	Method 1	Method 2
Muscle	0.075[a] (n=81)	0.096[a] (n=78)
	(P>0.15)	(0.05<P<0.10)
Liver	0.105[a] (n=74)	0.957[b] (n=49)
	(P<0.05)	(0.10<P<0.15)

[a] Kolmogorov-Smirnov Statistics since n>50.

[b] Shapiro-Wilk Statistic since n<50.

Table 2 contains the estimates of the mean and standard deviation of the transformed (log) variables for both variables and methods. As can be seen from this table, the Method 2 estimates tend to produce biased estimates for the mean (bias tends to produce larger estimates) and standard deviation (bias tends to produce smaller estimates). For the muscle data, the bias may have no practical significance. However, for the liver data this bias introduced in the estimated standard deviation seems substantial.

Table 2. MEAN AND STANDARD DEVIATION FOR TRANSFORMED VARIABLES

Tissue		Method 1	Method 2
Muscle	n	81	78
	mean	3.180	3.254
	std.dev.	0.887	0.817
Liver	n	74	49
	mean	2.251	2.651
	std.dev.	0.856	0.650

The raw data on the ^{137}Cs content of 1983 Road-Killed deer from which the above statistical analysis was made was taken from an ORNL document dated February 10, 1984. In this document are listed the results for high-resolution δ-ray analyses of the 1983 road-killed deer at the Oak Ridge National Laboratory expressed in units of pCi/kg (fresh weight).

Dr. John Beauchamp
Engineering Physics and Mathematics Division
Oak Ridge National Laboratory

APPENDIX C

FIELD COLLECTION ACTIVITIES AT THE ALABAMA ARMY AMMUNITION SITE

INTRODUCTION

The objective of this portion of the study (Phase II, Task 4) was to obtain samples to accurately describe the current concentrations of trinitrotoluene (TNT) and its metabolites in edible wildlife from the Alabama Army Ammunition Plant (AAAP), Childersburg, Alabama. As indicated in Quarterly Technical Progress Reports Nos. 3 and 4, the species of edible wildlife collected for analysis were deer, rabbit, and quail.

FIELD ACTIVITIES

A team of six personnel spent a total of ten days, from November 26, 1989 through December 5, 1989, collecting samples from the AAAP Site (Exhibit 1). The team consisted of Dr. Dennis E. Jones, Veterinary Toxicologist, ATSDR; Dr. Phillip M. Allred, Environmental Toxicologist, ATSDR; Ronnie Wynn, Site Manager, AAAP; Morgan Downing, consultant, ORNL; Wayne Downing, consultant, ORNL, and Dean Downing, consultant, ORNL. The sample collection period coincided with the normal open hunting season for all of the species collected. All the deer collected were taken by rifle; all rabbits and quail were taken by shotgun.

All animals collected were assigned an alphanumeric identification number corresponding to the specific year and day of collection, species, and order of collection during a specific day. The first digit of each assigned identification number was a 9, corresponding to 1989, the year collected. The next three digits assigned corresponded to the Julian Date of collection; e.g., 332 for November 28, 1989. The fifth digit assigned was a letter to designate the species, as follows:

D = deer

R = rabbit

Q = quail

The final (sixth) digit assigned corresponded to the order of collection of that species during that particular day; e.g., if the last digit of the sample identifier was a 3, this would indicate that this was the third specimen of that particular species collected on that day. As an example, the identifier 9332D2 would mean that this specimen was the second deer collected on November 28, 1989.

Tissue samples from specimen number 9332D2 were collected directly in the field; however, this proved difficult and time-consuming, and was found to increase the probability of sample contamination. Therefore, all other animals were brought to the central camp and processed (tissue samples collected) as soon as possible. The maximum time between field collection of specimens and commencement of tissue sample collection was estimated to be 1.5 hours.

Initial processing of samples consisted of assigning a sample identification number, noting and recording the sample collection point (see attached map), sex, whole body weight, general condition, appearance, and reproductive state. In addition, for deer only, the age was also noted (by analysis of dentition) and recorded.

For deer, triplicate 100 gram samples (approximate weight) of muscle and liver were then collected using stainless steel scalpel and tissue forceps. When animal conditions permitted, 10 ml samples of blood (approximate volume) and/or 10-gram samples of brain (approximate weight) were also collected.

For rabbits and quail, 10-gram muscle samples (approximate weight) were collected. As with the deer, when the condition of the animal permitted, 10-gram/ml samples of liver, brain, and/or blood were also collected.

Each individual muscle and liver sample was double wrapped in aluminum foil, the sample identification number was marked on the foil with permanent ink marker, and the sample was then placed in a sealed plastic bag along with a sample identification card, also marked in permanent ink. All brain and blood samples were placed in individual sealed glass scintillation vials, labeled accordingly with permanent ink marker, and then placed in sealed plastic bags along with a sample identification card. In addition, each individual sample and sample identification card was also marked to indicate the tissue sample type (Muscle, Liver, Brain, Blood). All samples were then stored on dry ice in igloo ice chests until completion of the field activities.

Dry ice was provided at the start of the studies on November 26, 1989, and replenished on December 1, 1989 and December 4, 1989.

CHAIN OF CUSTODY

All field sample collection activities were supervised by Dennis E. Jones, ATSDR. All field records were maintained by Dr. Jones, with a custody form completed for each animal specimen collected during the field activities portion of this study. The completed custody forms and tissue samples remained under the custody of Dr. Jones until delivered over to Dr. Lee R. Shugart, Environmental Sciences Division, ORNL, on December 6, 1989.

ADDITIONAL COMMENTS

The total number of animal specimens collected during the field activities were less than the optimal number planned for. However, a various conditions existed at this site which made the collection activities more difficult than originally anticipated and thereby merit mentioning.

The first condition that adversely impacted the ease of sample collection was the existence of a large number of predator species on site. These predators consisted of a large number coyotes, foxes, bobcats, and especially, wild (feral) dogs and were seen frequently during the field activities, particularly wild dogs. This large number of predators had somewhat depleted the small game population (quail, rabbits) to less than was anticipated, and also made those remaining animals extremely wary to hunting by dogs, and therefore much more difficult to collect.

The second condition encountered on site which may have impacted the ease of sample collection was the existence of trespassers. Four trespassing hunters were observed on the site during the field activities, and three of these trespassers were reported to and subsequently apprehended by the local game warden. The existence of such trespassers has undoubtedly impacted the on-site deer population, not only by reducing the total herd number, but also by increasing their wariness toward hunting, and thus the ease of collection for this study.

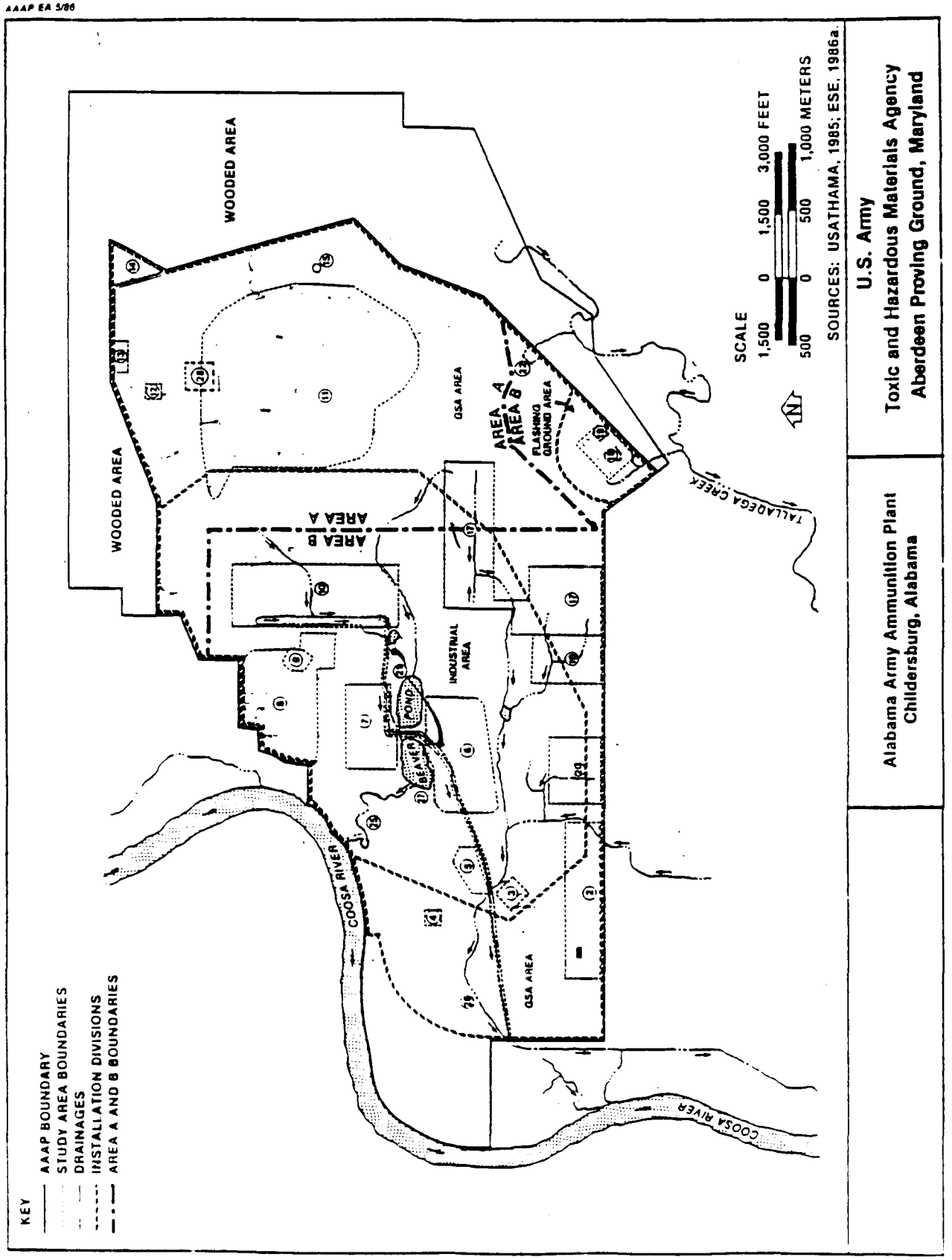
The final site condition which affected the sample collection activities was the type of site cover. A great deal of the site consists of either cut-over timber land or open land that has been allowed to grow back in a natural reforestation state. This has produced a site cover with an extremely thick, dense vegetative undergrowth, which in many places is almost impenetrable.

Although these conditions produce a favorable cover for harboring deer, quail, and rabbits, they also limited collection of these species to the less densely vegetated field borders and open areas.

Dennis E. Jones
Staff Toxicologist
Agency for Toxic Substances and Disease Registry

Appendix C

Exhibit 1



APPENDIX D

SAMPLE ANALYSES OF FIELD-COLLECTED SAMPLES

A total of 168 samples were processed and Summary Sheets for the determination of TNT and metabolites are available from the Project Officer upon request.

Sample Summary Sheets are attached to this Appendix. See Appendix C for an explanation of sample name (alphanumerical identification) for AAAP-collected animals.

The listing of specific results on each Summary Sheet includes the actual concentration observed in the extract, (column 1); the actual tissue concentration to which the measured extract concentration corresponds, (column 3); and the tissue concentration corrected for recovery, (column 4). In addition, the detection limits determined by the method of Hubaux and Vos, (1970), are listed in column 6. Any given result may have one, two, or three asterisks, (*), after the entry in column 3. These asterisks refer to footnotes included with that particular sample. However, the user of these results should note that as the number of asterisks increases the reliability of the data becomes less certain. Thus one asterisk,(*), generally indicates that a peak was found below the detection limit at the proper retention volume but the absorbance under this peak was not great enough to confirm this analyte by spectral matching. Two asterisks (**) indicate that although a peak occurred at the proper retention volume, there appeared to be an interference peculiar to that extract and the spectrum of the absorbance under that peak could not be matched with the spectrum of the authentic compound. Three asterisks indicates that a peak occurred at a retention volume near, (but not at), that expected for the target analyte, (too close to be completely ignored); however no spectral confirmation could be ascertained. Thus the probability of a result marked with *** being caused by the target analyte is very low. The notations made on the results can be summarized as follows:

	Spectral Match	Interference from extract	Retention Volume Match
*	No	No	Yes
**	No	Yes	Yes
***	No	Yes or No	No

John Caton, Ph.D.

Supplement to Appendix D

Summary Sheets Packet (a): Deer Liver

REPORT OF TNT METABOLITE ANALYSES

CDL3A849
DEER LIVER
CONTROL
JECA849

COLUMN:	C18/ANION	COL LNG, MM:	150
FLOW, ML/MIN:	1	PRESSURE, BAR	173
INJ VOL, UL:	50	VD TIME, MIN:	1.82
VEL, MM/SEC:	1.37362		

SOLVENT A: PHOSPHATE AT 0.015 M AND PH 5.14 IN 90/10 WATER/MEOH
SOLVENT B: METHANOL
SOLVENT C: ACETONITRILE

SOLVENT PROGRAM

TIME	% A	% B	% C	FLOW ML/M
1	72	0	28	1
5	68	0	32	1
14	68	0	32	1
20	44	12	44	1
26	2	54	44	1
33	2	54	44	1
38	72	0	28	1
45	72	0	28	1

SAMPLE NAME:	CON D L3 A849	RECOVERY STD:	1,3-DINITROBENZENE
WEIGHT,GMS:	2	CONCEN, PPM:	25
EXT VOL, UL:	500	VOLUME ADDED:	100
		NG ADDED:	2500
% RECOVERY:	66.35	NG FOUND:	1658.

COMPOUND	CONC PPM (1)	AREA (2)	PPM IN TISSUE MEAS (3)		CORR. (4)	RES F (5)	DET L PPM (6)
1,3-DINITROBENZENE--RS	3.32	490	0.83		1.25	3.77	0.05
2,4,6-TRINITROTOLUENE	0.08	7.3	0.02	**	0.03	11.63	0.05
1,3,5-TRINITROBENZENE	0.54	50	0.14	***	0.20	10.8	0.05
2,4-DIAMINO-6-NITROTOLUENE	0.28	11	0.07	**	0.10	25.25	0.18
2,6-DIAMINO-4-NITROTOLUENE	0.19	19	0.05	**	0.07	10	0.05
4-AMINO-2,6-DINITROTOLUENE	0.00	0	0.00		0.00	13.55	0.15
2-AMINO-4,6-DINITROTOLUENE	0.02	1.6	0.00	**	0.01	10.62	0.06
4-HYDROXYLAMINO-2,6-DNT	0.05	2.3	0.01	**	0.02	21	0.75
TRINITROBENZYL ALCOHOL	0.00	0	0.00		0.00	30.12	0.13
TRINITROBENZOIC ACID	0.00	0	0.00		0.00	22.7	0.1
TETRANITROAZOXYTOLUENE	0.02	3.2	0.00	**	0.01	5.53	0.67

NOTES: (1) CONCENTRATION IN EXTRACT; 1:1:3 MEOH:CH3CN:BUFFER
(2) PEAK AREA IN CHROMATOGRAM
(3) CONCENTRATION IN TISSUE WITH NO RECOVERY CORRECTION
(4) CONCENTRATION IN TISSUE BASED ON RECOVERY OF DNB SPIKE
(5) RESPONSE FACTOR FOR A GIVEN COMPONENT, PPB/AREA UNIT
(6) DETECTION LIMIT FOR MEASURED CONCENTRATION IN TISSUE; VALUES FOR MEASURED CONCENTRATION IN TISSUE FALLING BELOW THIS LEVEL ARE NOT RELIABLE, (PROBABLY INSIGNIFICANT?
* SPECTRA IS INCONCLUSIVE IN CONFIRMING THIS COMPOUND
** APPEARS TO BE AN INTERFERENCE IN THE EXTRAC
*** RETENTION VOLUME MATCH IS MARGINAL

CHECKED BY: John Caton DATE 5-2-96

No compound can be confirmed above the detection limit, etc.

R

REPORT OF SPIKE RECOVERY D3L49336SPIKE
SPK58A25

COLUMN:	C18/ANION	COL LNG, MM:	150
FLOW, ML/MIN:	1	PRESSURE, BAR	173
INJ VOL, UL:	50	VD TIME, MIN:	1.82
VEL, MM/SEC:	1.37[illegible]62		

SOLVENT A: PHOSPHATE AT 0.015 M AND PH 5.14 IN 90/10 WATER/MEOH
SOLVENT B: METHANOL
SOLVENT C: ACETONITRILE

SOLVENT PROGRAM

TIME	% A	% B	% C	FLOW ML/M
1	72	0	28	1
5	68	0	32	1
14	68	0	32	1
20	44	12	44	1
26	2	54	44	1
33	2	54	44	1
38	72	0	28	1
45	72	0	28	1

SAMPLE NAME:	9336D3L4SPK	SPIKE ADDED: CONCEN, PPM:	25
EXT VOL, UL:	500	VOLUME ADDED:	100

% RECOVERY: 68.41

SPIKESHOULD CONTAIN 5 PPM***

COMPOUND	CONC PPM (1)	AREA (2)	CONC XPT (3)		% REC (4)	RES F (5)
1,3-DINITROBENZENE--RS	3.42	495	5.00		68.41	6.91
2,4,6-TRINITROTOLUENE	4.17	393	5.00		83.32	10.6
1,3,5-TRINITROBENZENE	4.09	379	5.00		81.86	10.8
2,4-DIAMINO-6-NITROTOLUENE	0.83	34.4	5.00		16.51	24
2,6-DIAMINO-4-NITROTOLUENE	2.00	207	5.00		39.95	9.65
4-AMINO-2,6-DINITROTOLUENE	4.07	291	5.00		81.48	14
2-AMINO-4,6-DINITROTOLUENE	4.13	393	5.00		82.53	10.5
4-HYDROXYLAMINO-2,6-DNT	0.00	0	5.00	*	0.00	21
TRINITROBENZYL ALCOHOL	0.28	9.3	5.00	*	5.60	30.1
TRINITROBENZOIC ACID	0.00	0	5.00	*	0.00	22.7
TETRANITROAZOXYTOLUENE	0.00	0	5.00	**	0.00	5.53

NOTES: (1) CONCENTRATION FOUND IN SPIKED SAMPLE
(2) PEAK AREA IN CHROMATOGRAM
(3) CONCENTRATION EXPECTED IN SAMPLE FOR 100 % RECOVERY
(4) PERCENT RECOVERY OF SPIKE
(5) RESPONSE FACTOR FOR A GIVEN COMPONENT, PPB/AREA UNIT
* COMPONENT NOT ADDED
** COMPONENT NOT RECOVERED--POSSIBLE DECOMPOSITION

CHECKED BY: John Caton DATE 5-1-90

R

REPORT OF TNT METABOLITE ANALYSES D2L19332

COLUMN:	C18/ANION	COL LNG, MM:	150
FLOW, ML/MIN:	1	PRESSURE, BAR	173
INJ VOL, UL:	50	VD TIME, MIN:	1.82
VEL, MM/SEC:	1.37362		
SOLVENT A:	PHOSPHATE AT 0.015 M AND PH 5.14 IN 90/10 WATER/MEOH		
SOLVENT B:	METHANOL		
SOLVENT C:	ACETONITRILE		

SOLVENT PROGRAM

TIME	% A	% B	% C	FLOW ML/M
1	72	0	28	1
5	68	0	32	1
14	68	0	32	1
20	44	12	44	1
26	2	54	44	1
33	2	54	44	1
38	72	0	28	1
45	72	0	28	1

SAMPLE NAME:	9332D2L1	RECOVERY STD:	1,3-DINITROBENZENE
WEIGHT,GMS:	2.01	CONCEN, PPM:	25
EXT VOL, UL:	500	VOLUME ADDED:	100
		NG ADDED:	2500
% RECOVERY:	51.55	NG FOUND:	1288.

COMPOUND	CONC PPM (1)	AREA (2)	PPM IN TISSUE MEAS (3)		CORR. (4)	RES F (5)	DET L PPM (6)
1,3-DINITROBENZENE--RS	2.58	373	0.64		1.24	6.91	0.05
2,4,6-TRINITROTOLUENE	0.03	3	0.01	*	0.01	9.72	0.05
1,3,5-TRINITROBENZENE	0.08	7.2	0.02	*	0.04	10.8	0.05
2,4-DIAMINO-6-NITROTOLUENE	0.00	0	0.00		0.00	22.6	0.18
2,6-DIAMINO-4-NITROTOLUENE	0.10	11	0.03	**	0.05	9.18	0.05
4-AMINO-2,6-DINITROTOLUENE	0.04	3.1	0.01	*	0.02	14	0.15
2-AMINO-4,6-DINITROTOLUENE	0.05	5	0.01	*	0.02	10.1	0.06
4-HYDROXYLAMINO-2,6-DNT	0.00	0	0.00		0.00	21	0.75
TRINITROBENZYL ALCOHOL	0.00	0	0.00		0.00	25	0.13
TRINITROBENZOIC ACID	0.00	0	0.00		0.00	22.7	0.1
TETRANITROAZOXYTOLUENE	0.00	0	0.00		0.00	5.53	0.67

NOTES: (1) CONCENTRATION IN EXTRACT; 1:1:3 MEOH:CH3CN:BUFFER
(2) PEAK AREA IN CHROMATOGRAM
(3) CONCENTRATION IN TISSUE WITH NO RECOVERY CORRECTION
(4) CONCENTRATION IN TISSUE BASED ON RECOVERY OF DNB SPIKE
(5) RESPONSE FACTOR FOR A GIVEN COMPONENT, PPB/AREA UNIT
(6) DETECTION LIMIT FOR MEASURED CONCENTRATION IN TISSUE: VALUES FOR MEASURED CONCENTRATION IN TISSUE FALLING BELOW THIS LEVEL ARE NOT RELIABLE. (PROBABLY INSIGNIFICANT?)
* SPECTRA IN INCONCLUSIVE IN CONFIRNING THIS COMPOUND
** APPEARS TO BE AN INTERFERENCE IN THE EXTRACT

CHECKED BY: John Caton DATE 5-1-90

Nothing above detection limits QCC

Supplement to Appendix D

Summary Sheets Packet (b): Deer Muscle

REPORT OF SPIKE RECOVERY

SPK59A36
CONTROL D1 M4
SPIKED AT 5 PPM
892-20-1

COLUMN:	C18/ANION	COL LNG, MM:	150
FLOW, ML/MIN:	1	PRESSURE, BAR	166
INJ VOL, UL:	50	VD TIME, MIN:	1.78
VEL, MM/SEC:	1.40449		

SOLVENT A: PHOSPHATE AT 0.015 M AND PH 5.14 IN 90/10 WATER/MEOH
SOLVENT B: METHANOL
SOLVENT C: ACETONITRILE

SOLVENT PROGRAM

TIME	% A	% B	% C	FLOW ML/M
1	72	0	28	1
5	68	0	32	1
14	68	0	32	1
20	44	12	44	1
26	2	54	44	1
33	2	54	44	1
38	72	0	28	1
45	72	0	28	1

SAMPLE NAME:	CONTROL D1M4	SPIKE ADDED:	
		CONCEN, PPM:	25
EXT VOL, UL:	500	VOLUME ADDED:	100

% RECOVERY: 65.80

SPIKESHOULD CONTAIN 5 PPM***

COMPOUND	CONC PPM (1)	AREA (2)	CONC XPT (3)		% REC (4)	RES F (5)
1,3-DINITROBENZENE--RS	3.29	486	5.00		65.8	6.77
2,4,6-TRINITROTOLUENE	5.04	433	5.00		100.7	11.63
1,3,5-TRINITROBENZENE	2.85	264	5.00		57.0	10.8
2,4-DIAMINO-6-NITROTOLUENE	2.37	94	5.00		47.5	25.25
2,6-DIAMINO-4-NITROTOLUENE	3.51	351	5.00		70.2	10
4-AMINO-2,6-DINITROTOLUENE	3.22	238	5.00		64.5	13.55
2-AMINO-4,6-DINITROTOLUENE	3.82	360	5.00		76.5	10.62
4-HYDROXYLAMINO-2,6-DNT	0.48	23	5.00	*	9.7	21
TRINITROBENZYL ALCOHOL	0.00	0	5.00	*	0.0	30.12
TRINITROBENZOIC ACID	0.00	0	5.00	*	0.0	22.7
TETRANITROAZOXYTOLUENE	0.51	93	5.00	***	10.3	5.53

NOTES: (1) CONCENTRATION FOUND IN SPIKED SAMPLE
(2) PEAK AREA IN CHROMATOGRAM
(3) CONCENTRATION EXPECTED IN SAMPLE FOR 100 % RECOVERY
(4) PERCENT RECOVERY OF SPIKE
(5) RESPONSE FACTOR FOR A GIVEN COMPONENT, PPB/AREA UNIT
* COMPONENT NOT ADDED

*** LOW RECOVERY--POSSIBLE DECOMPOSITION

REPORTED BY: John Caton DATE 5-3-90

R

REPORT OF TNT METABOLITE ANALYSES CD1M1933
CONTROL

COLUMN:	C18/ANION	COL LNG, MM:	150
FLOW, ML/MIN:	1	PRESSURE, BAR	166
INJ VOL, UL:	50	VD TIME, MIN:	1.78
VEL, MM/SEC:	1.40449		

SOLVENT A: PHOSPHATE AT 0.015 M AND PH 5.14 IN 90/10 WATER/MEOH
SOLVENT B: METHANOL
SOLVENT C: ACETONITRILE

SOLVENT PROGRAM

TIME	% A	% B	% C	FLOW ML/M
1	72	0	28	1
5	68	0	32	1
14	68	0	32	1
20	44	12	44	1
26	2	54	44	1
33	2	54	44	1
38	72	0	28	1
45	72	0	28	1

SAMPLE NAME:	CONTROL D1M1	RECOVERY STD:	1,3-DINITROBENZENE
WEIGHT,GMS:	2.01	CONCEN, PPM:	25
EXT VOL, UL:	500	VOLUME ADDED:	100
		NG ADDED:	2500
% RECOVERY:	73.66	NG FOUND:	1841.

COMPOUND	CONC PPM (1)	AREA (2)	PPM IN TISSUE MEAS (3)		CORR. (4)	RES F (5)	DET L PPM (6)
1,3-DINITROBENZENE--RS	3.68	533	0.92		1.24	6.91	0.05
2,4,6-TRINITROTOLUENE	0.26	23	0.07	**	0.09	11.49	0.05
1,3,5-TRINITROBENZENE	0.05	4.4	0.01	**	0.02	10.8	0.05
2,4-DIAMINO-6-NITROTOLUENE	0.07	3	0.02	**	0.02	22.6	0.18
2,6-DIAMINO-4-NITROTOLUENE	0.03	2.8	0.01	**	0.01	9.18	0.05
4-AMINO-2,6-DINITROTOLUENE	0.00	0	0.00		0.00	12.9	0.15
2-AMINO-4,6-DINITROTOLUENE	0.00	0	0.00		0.00	9.26	0.06
4-HYDROXYLAMINO-2,6-DNT	0.00	0	0.00		0.00	21	0.75
TRINITROBENZYL ALCOHOL	0.05	1.9	0.01	**	0.02	25.1	0.13
TRINITROBENZOIC ACID	0.00	0	0.00		0.00	22.7	0.1
TETRANITROAZOXYTOLUENE	0.03	4.35	0.01	**	0.01	6.9	0.67

NOTES: (1) CONCENTRATION IN EXTRACT; 1:1:3 MEOH:CH3CN:BUFFER
(2) PEAK AREA IN CHROMATOGRAM
(3) CONCENTRATION IN TISSUE WITH NO RECOVERY CORRECTION
(4) CONCENTRATION IN TISSUE BASED ON RECOVERY OF DNB SPIKE
(5) RESPONSE FACTOR FOR A GIVEN COMPONENT, PPB/AREA UNIT
(6) DETECTION LIMIT FOR MEASURED CONCENTRATION IN TISSUE; VALUES FOR MEASURED CONCENTRATION IN TISSUE FALLING BELOW THIS LEVEL ARE NOT RELIABLE. (PROBABLY INSIGNIFICANT?)
* SPECTRA IS INCONCLUSIVE IN CONFIRMING THIS COMPOUND
** APPEARS TO BE AN INTERFERENCE IN THE EXTRACT
*** RETENTION VOLUME MATCH IS MARGINAL

CHECKED BY: John Caton DATE 5-3-90

Note Controls look like samples!
No confirmation of anything above the detection limit

R

REPORT OF TNT METABOLITE ANALYSES D3M19332

COLUMN:	C18/ANION	COL LNG, MM:	150
FLOW, ML/MIN:	1	PRESSURE, BAR	160
INJ VOL, UL:	50	VD TIME, MIN:	1.93
VEL, MM/SEC:	1.29533		
SOLVENT A:	PHOSPHATE AT 0.015 M AND PH 5.14 IN 90/10 WATER/MEOH		
SOLVENT B:	METHANOL		
SOLVENT C:	ACETONITRILE		

SOLVENT PROGRAM

TIME	% A	% B	% C	FLOW ML/M
1	72	0	28	1
5	68	0	32	1
14	68	0	32	1
20	44	12	44	1
26	2	54	44	1
33	2	54	44	1
38	72	0	28	1
45	72	0	28	1

SAMPLE NAME:	9332 D3 M1	RECOVERY STD:	1,3-DINITROBENZENE
WEIGHT,GMS:	2	CONCEN, PPM:	25
EXT VOL, UL:	500	VOLUME ADDED:	100
		NG ADDED:	2500
% RECOVERY:	52.54	NG FOUND:	1313.

COMPOUND	CONC PPM (1)	AREA (2)	PPM IN TISSUE MEAS (3)		PPM IN TISSUE CORR. (4)	RES F (5)	DET L PPM (6)
1,3-DINITROBENZENE--RS	2.63	388	0.66		1.25	6.77	0.05
2,4,6-TRINITROTOLUENE	0.13	11	0.03	*	0.06	11.63	0.05
1,3,5-TRINITROBENZENE	0.00	0	0.00		0.00	10.8	0.05
2,4-DIAMINO-6-NITROTOLUENE	0.13	5.2	0.03	*	0.06	25.25	0.18
2,6-DIAMINO-4-NITROTOLUENE	0.04	4.2	0.01	*	0.02	10	0.05
4-AMINO-2,6-DINITROTOLUENE	0.00	0	0.00		0.00	13.55	0.15
2-AMINO-4,6-DINITROTOLUENE	0.00	0	0.00		0.00	10.62	0.06
4-HYDROXYLAMINO-2,6-DNT	0.10	4.8	0.03	*	0.05	21	0.75
TRINITROBENZYL ALCOHOL	0.84	28	0.21	***	0.40	30.12	0.13
TRINITROBENZOIC ACID	0.00	0	0.00		0.00	22.7	0.1
TETRANITROAZOXYTOLUENE	0.00	0	0.00		0.00	5.53	0.67

NOTES: (1) CONCENTRATION IN EXTRACT; 1:1:3 MEOH:CH3CN:BUFFER
(2) PEAK AREA IN CHROMATOGRAM
(3) CONCENTRATION IN TISSUE WITH NO RECOVERY CORRECTION
(4) CONCENTRATION IN TISSUE BASED ON RECOVERY OF DNB SPIKE
(5) RESPONSE FACTOR FOR A GIVEN COMPONENT, PPB/AREA UNIT
(6) DETECTION LIMIT FOR MEASURED CONCENTRATION IN TISSUE: VALUES FOR MEASURED CONCENTRATION IN TISSUE FALLING BELOW THIS LEVEL ARE NOT RELIABLE. (PROBABLY INSIGNIFICANT)
* SPECTRA IS INCONCLUSIVE IN CONFIRMING THIS COMPOUND
** APPEARS TO BE AN INTERFERENCE IN THE EXTRACT
*** RETENTION VOLUME MATCH IS MARGINAL

CHECKED BY: John Caton DATE 5-3-90

Supplement to Appendix D

Summary Sheets Packet (c): Rabbit

R

REPORT OF TNT METABOLITE ANALYSES R1L29334

COLUMN:	C18/ANION	COL LNG, MM:	150
FLOW, ML/MIN:	1	PRESSURE, BAR	166
INJ VOL, UL:	50	VD TIME, MIN:	1.78
VEL, MM/SEC:	1.40449		
SOLVENT A:	PHOSPHATE AT 0.015 M AND PH 5.14 IN 90/10 WATER/MEOH		
SOLVENT B:	METHANOL		
SOLVENT C:	ACETONITRILE		

SOLVENT PROGRAM

TIME	% A	% B	% C	FLOW ML/M
1	72	0	28	1
5	68	0	32	1
14	68	0	32	1
20	44	12	44	1
26	2	54	44	1
33	2	54	44	1
38	72	0	28	1
45	72	0	28	1

SAMPLE NAME:	9334 R1 L2	RECOVERY STD:	1,3-DINITROBENZENE
WEIGHT,GMS:	2.01	CONCEN, PPM:	25
EXT VOL, UL:	500	VOLUME ADDED:	100
		NG ADDED:	2500
% RECOVERY:	49.20	NG FOUND:	1229.

COMPOUND	CONC PPM (1)	AREA (2)	PPM IN TISSUE MEAS (3)		CORR. (4)	RES F (5)	DET L PPM (6)
1,3-DINITROBENZENE--RS	2.46	356	0.61		1.24	6.91	0.05
2,4,6-TRINITROTOLUENE	0.00	0	0.00		0.00	11.49	0.05
1,3,5-TRINITROBENZENE	0.00	0	0.00		0.00	10.8	0.05
2,4-DIAMINO-6-NITROTOLUENE	0.00	0	0.00		0.00	22.6	0.18
2,6-DIAMINO-4-NITROTOLUENE	0.21	23	0.05	***	0.11	9.18	0.05
4-AMINO-2,6-DINITROTOLUENE	0.00	0	0.00		0.00	12.9	0.15
2-AMINO-4,6-DINITROTOLUENE	0.00	0	0.00		0.00	9.26	0.06
4-HYDROXYLAMINO-2,6-DNT	0.05	2.3	0.01	*	0.02	21	0.75
TRINITROBENZYL ALCOHOL	0.45	18	0.11	***	0.23	25.1	0.13
TRINITROBENZOIC ACID	0.00	0	0.00		0.00	22.7	0.1
TETRANITROAZOXYTOLUENE	0.01	1.2	0.00	*	0.00	6.9	0.67

NOTES: (1) CONCENTRATION IN EXTRACT; 1:1:3 MEOH:CH3CN:BUFFER
(2) PEAK AREA IN CHROMATOGRAM
(3) CONCENTRATION IN TISSUE WITH NO RECOVERY CORRECTION
(4) CONCENTRATION IN TISSUE BASED ON RECOVERY OF DNB SPIKE
(5) RESPONSE FACTOR FOR A GIVEN COMPONENT, PPB/AREA UNIT
(6) DETECTION LIMIT FOR MEASURED CONCENTRATION IN TISSUE; VALUES FOR MEASURED CONCENTRATION IN TISSUE FALLING BELOW THIS LEVEL ARE NOT RELIABLE, (PROBABLY INSIGNIFICANT?)
* SPECTRA IS INCONCLUSIVE IN CONFIRMING THIS COMPOUND
** APPEARS TO BE AN INTERFERENCE IN THE EXTRACT
*** RETENTION VOLUME MATCH IS MARGINAL

CHECKED BY: John Caton DATE 5-3-90

Can't not confirm anything above the detection limits

R

REPORT OF SPIKE RECOVERY

SPK59A32
9334 R1 M4
SPIKED AT 5 PPM
892-20-1

COLUMN:	C18/ANION	COL LNG, MM:	150
FLOW, ML/MIN:	1	PRESSURE, BAR	166
INJ VOL, UL:	50	VD TIME, MIN:	1.78
VEL, MM/SEC:	1.40449		

SOLVENT A: PHOSPHATE AT 0.015 M AND PH 5.14 IN 90/10 WATER/MEOH
SOLVENT B: METHANOL
SOLVENT C: ACETONITRILE

SOLVENT PROGRAM

TIME	% A	% B	% C	FLOW ML/M
1	72	0	28	1
5	68	0	32	1
14	68	0	32	1
20	44	12	44	1
26	2	54	44	1
33	2	54	44	1
38	72	0	28	1
45	72	0	28	1

SAMPLE NAME:	9334 R1M4 SP	SPIKE ADDED: CONCEN, PPM:	25
EXT VOL, UL:	500	VOLUME ADDED:	100

% RECOVERY: 57.41

SPIKESHOULD CONTAIN 5 PPM***

COMPOUND	CONC PPM (1)	AREA (2)	CONC XPT (3)		% REC (4)	RES F (5)
1,3-DINITROBENZENE--RS	2.87	424	5.00		57.4	6.77
2,4,6-TRINITROTOLUENE	4.09	352	5.00		81.9	11.63
1,3,5-TRINITROBENZENE	3.08	285	5.00		61.6	10.8
2,4-DIAMINO-6-NITROTOLUENE	2.53	100	5.00		50.5	25.25
2,6-DIAMINO-4-NITROTOLUENE	3.65	365	5.00		73.0	10
4-AMINO-2,6-DINITROTOLUENE	3.22	238	5.00		64.5	13.55
2-AMINO-4,6-DINITROTOLUENE	4.24	399	5.00		84.7	10.62
4-HYDROXYLAMINO-2,6-DNT	0.25	12	5.00	*	5.0	21
TRINITROBENZYL ALCOHOL	0.26	8.6	5.00	*	5.2	30.12
TRINITROBENZOIC ACID	0.00	0	5.00	*	0.0	22.7
TETRANITROAZOXYTOLUENE	0.21	38	5.00	***	4.2	5.53

NOTES: (1) CONCENTRATION FOUND IN SPIKED SAMPLE
(2) PEAK AREA IN CHROMATOGRAM
(3) CONCENTRATION EXPECTED IN SAMPLE FOR 100 % RECOVERY
(4) PERCENT RECOVERY OF SPIKE
(5) RESPONSE FACTOR FOR A GIVEN COMPONENT, PPB/AREA UNIT
* COMPONENT NOT ADDED

*** LOW RECOVERY--POSSIBLE DECOMPOSITION

CHECKED BY: John Cator DATE 5-4-90

R

REPORT OF TNT METABOLITE ANALYSES

CR1M1949
JEC9A49A
CONTROL

COLUMN:	C18/ANION	COL LNG, MM:	150
FLOW, ML/MIN:	1	PRESSURE, BAR	177
INJ VOL, UL:	50	VD TIME, MIN:	1.94
VEL, MM/SEC:	1.28865		
SOLVENT A:	PHOSPHATE AT 0.015 M AND PH 5.14 IN 90/10 WATER/MEOH		
SOLVENT B:	METHANOL		
SOLVENT C:	ACETONITRILE		

SOLVENT PROGRAM

TIME	% A	% B	% C	FLOW ML/M
1	72	0	28	1
5	68	0	32	1
14	68	0	32	1
20	44	12	44	1
24	2	54	44	1
27	2	54	44	1
29	72	0	28	1
33	72	0	28	1

SAMPLE NAME:	CONTROL R1 M1	RECOVERY STD:	1,3-DINITROBENZENE
WEIGHT,GMS:	2.01	CONCEN, PPM:	25
EXT VOL, UL:	500	VOLUME ADDED:	100
		NG ADDED:	2500
% RECOVERY:	56.60	NG FOUND:	1415.

COMPOUND	CONC PPM (1)	AREA (2)	PPM IN TISSUE MEAS (3)		PPM IN TISSUE CORR. (4)	RES F (5)	DET L PPM (6)
1,3-DINITROBENZENE--RS	2.83	529	0.70		1.24	5.35	0.05
2,4,6-TRINITROTOLUENE	0.69	57	0.17	**	0.30	12.05	0.05
1,3,5-TRINITROBENZENE	0.00	0	0.00		0.00	10.8	0.05
2,4-DIAMINO-6-NITROTOLUENE	0.12	5.7	0.03	***	0.05	20.93	0.18
2,6-DIAMINO-4-NITROTOLUENE	0.03	3.4	0.01	***	0.01	8	0.05
4-AMINO-2,6-DINITROTOLUENE	0.03	3	0.01		0.01	8.7	0.15
2-AMINO-4,6-DINITROTOLUENE	0.00	0	0.00		0.00	7.81	0.06
4-HYDROXYLAMINO-2,6-DNT	0.03	1.5	0.01	**	0.01	21	0.75
TRINITROBENZYL ALCOHOL	0.09	3.3	0.02	**	0.04	27.03	0.13
TRINITROBENZOIC ACID	0.00	0	0.00		0.00	23.69	0.1
TETRANITROAZOXYTOLUENE	0.05	7.6	0.01	**	0.02	6.9	0.67

NOTES: (1) CONCENTRATION IN EXTRACT; 1:1:3 MEOH:CH3CN:BUFFER
(2) PEAK AREA IN CHROMATOGRAM
(3) CONCENTRATION IN TISSUE WITH NO RECOVERY CORRECTION
(4) CONCENTRATION IN TISSUE BASED ON RECOVERY OF DNB SPIKE
(5) RESPONSE FACTOR FOR A GIVEN COMPONENT, PPB/AREA UNIT
(6) DETECTION LIMIT FOR MEASURED CONCENTRATION IN TISSUE: VALUES FOR MEASURED CONCENTRATION IN TISSUE FALLING BELOW THIS LEVEL ARE NOT RELIABLE. (PROBABLY INSIGNIFICANT?)
* SPECTRA IS INCONCLUSIVE IN CONFIRMING THIS COMPOUND
** APPEARS TO BE AN INTERFERENCE IN THE EXTRACT
*** RETENTION VOLUME MATCH IS MARGINAL

CHECKED BY: John Caton DATE 5-4-90

This extract produced a "lussy" chromatogram but there is no spectral confirmation for any target compound. Mtx. controls and samples are similar.

Supplement to Appendix D

Summary Sheets Packet (d): Quail

REPORT OF TNT METABOLITE ANALYSES Q1: 9334

COLUMN:	C18/ANION	COL LNG, MM:	150
FLOW, ML/MIN:	1	PRESSURE, BAR	177
INJ VOL, UL:	50	VD TIME, MIN:	1.94
VEL, MM/SEC:	1.28865		
SOLVENT A:	PHOSPHATE AT 0.015 M AND PH 5.14 IN 90/10 WATER/MEOH		
SOLVENT B:	METHANOL		
SOLVENT C:	ACETONITRILE		

SOLVENT PROGRAM

TIME	% A	% B	% C	FLOW ML/M
1	72	0	28	1
5	68	0	32	1
14	68	0	32	1
20	44	12	44	1
24	2	54	44	1
27	2	54	44	1
29	72	0	28	1
33	72	0	28	1

SAMPLE NAME:	9334 Q1 L	RECOVERY STD:	1,3-DINITROBENZENE
WEIGHT,GMS:	2	CONCEN, PPM:	25
EXT VOL, UL:	500	VOLUME ADDED:	100
		NG ADDED:	2500
% RECOVERY:	64.20	NG FOUND:	1605

COMPOUND	CONC PPM (1)	AREA (2)	PPM IN TISSUE MEAS (3)		CORR. (4)	RES F (5)	DET L PPM (6)
1,3-DINITROBENZENE--RS	3.21	600	0.80		1.25	5.35	0.05
2,4,6-TRINITROTOLUENE	0.25	21	0.06	**	0.10	12.05	0.05
1,3,5 TRINITROBENZENE	0.11	10.6	0.03	**	0.04	10.8	0.05
2,4-DIAMINO-6-NITROTOLUENE	0.00	0	0.00		0.00	20.83	0.18
2,6-DIAMINO-4-NITROTOLUENE	0.14	17	0.03	***	0.05	8	0.05
4-AMINO-2,6-DINITROTOLUENE	0.03	3.8	0.01	*	0.01	8.7	0.15
2-AMINO-4,6-DINITROTOLUENE	0.01	1.4	0.00	*	0.00	7.81	0.06
4-HYDROXYLAMINO-2,6-DNT	0.41	19.7	0.10	***	0.16	21	0.75
TRINITROBENZYL ALCOHOL	1.84	68	0.46	***	0.72	27.03	0.13
TRINITROBENZOIC ACID	5.64	238	1.41	***	2.20	23.69	0.1
TETRANITROAZOXYTOLUENE	0.08	12.3	0.02	*	0.03	6.9	0.67

NOTES: (1) CONCENTRATION IN EXTRACT; 1:1:3 MEOH:CH3CN:BUFFER
(2) PEAK AREA IN CHROMATOGRAM
(3) CONCENTRATION IN TISSUE WITH NO RECOVERY CORRECTION
(4) CONCENTRATION IN TISSUE BASED ON RECOVERY OF DNB SPIKE
(5) RESPONSE FACTOR FOR A GIVEN COMPONENT, PPB/AREA UNIT
(6) DETECTION LIMIT FOR MEASURED CONCENTRATION IN TISSUE; VALUES FOR MEASURED CONCENTRATION IN TISSUE FALLING BELOW THIS LEVEL ARE NOT RELIABLE, (PROBABLY INSIGNIFICANT?)
* SPECTRA IS INCONCLUSIVE IN CONFIRMING THIS COMPOUND
** APPEARS TO BE AN INTERFERENCE IN THE EXTRACT
*** MAJOR INTERFERENCE FROM EXTRACT

CHECKED BY: John Cator DATE 5-4-90

"Dirty Extract" Major interferences in areas where trinitrobenzyl alcohol and trinitrobenzoic acid chromatograph. However there is no spectral confirmation of these compounds. One would not expect to see that much acid if the trinitrobenzene were observed. Nothing can be confirmed above the detection limit.
JEC

REPORT OF SPIKE RECOVERY

SPK51A14
CONTROL Q1 M4
SPIKED AT 5 PPM
892-20-1

COLUMN:	C18/ANION	COL LNG, MM:	150
FLOW, ML/MIN:	1	PRESSURE, BAR	166
INJ VOL, UL:	50	VD TIME, MIN:	1.78
VEL, MM/SEC:	1.40449		

SOLVENT A: PHOSPHATE AT 0.015 M AND PH 5.14 IN 90/10 WATER/MEOH
SOLVENT B: METHANOL
SOLVENT C: ACETONITRILE

SOLVENT PROGRAM

TIME	% A	% B	% C	FLOW ML/M
1	72	0	28	1
5	68	0	32	1
14	68	0	32	1
20	44	12	44	1
24	2	54	44	1
27	2	54	44	1
29	72	0	28	1
33	72	0	28	1

SAMPLE NAME:	CONTROL	SPIKE ADDED:	
	SPIKE Q1 M4	CONC 1, PPM:	25
EXT VOL, UL:	500	VOLUME ADDED:	100

% RECOVERY: 49.56

SPIKESHOULD CONTAIN 5 PPM***

COMPOUND	CONC PPM (1)	AREA (2)	CONC XPT (3)		% REC (4)	RES F (5)
1,3-DINITROBENZENE--RS	2.48	366	5.00		49.6	6.77
2,4,6-TRINITROTOLUENE	3.69	317	5.00		73.7	11.63
1,3,5-TRINITROBENZENE	2.73	253	5.00		54.6	10.8
2,4-DIAMINO-6-NITROTOLUENE	1.79	71	5.00		35.9	25.25
2,6-DIAMINO-4-NITROTOLUENE	2.66	266	5.00		53.2	10
4-AMINO-2,6-DINITROTOLUENE	2.67	197	5.00		53.4	13.55
2-AMINO-4,6-DINITROTOLUENE	3.08	290	5.00		61.6	10.62
4-HYDROXYLAMINO-2,6-DNT	0.00	0	5.00	*	0.0	21
TRINITROBENZYL ALCOHOL	0.54	18	5.00	*	10.8	30.12
TRINITROBENZOIC ACID	0.00	0	5.00	*	0.0	22.7
TETRANITROAZOXYTOLUENE	0.34	62	5.00	***	6.9	5.53

NOTES: (1) CONCENTRATION FOUND IN SPIKED SAMPLE
(2) PEAK AREA IN CHROMATOGRAM
(3) CONCENTRATION EXPECTED IN SAMPLE FOR 100 % RECOVERY
(4) PERCENT RECOVERY OF SPIKE
(5) RESPONSE FACTOR FOR A GIVEN COMPONENT, PPB/AREA UNIT
* COMPONENT NOT ADDED

*** LOW RECOVERY--POSSIBLE DECOMPOSITION

CHECKED BY: Sabra Caton DATE 5-4-90

R

REPORT OF TNT METABOLITE ANALYSES Q1M19334

COLUMN:	C18/ANION	COL LNG, MM:	150
FLOW, ML/MIN:	1	PRESSURE, BAR	177
INJ VOL, UL:	50	VD TIME, MIN:	1.94
VEL, MM/SEC:	1.28865		
SOLVENT A:	PHOSPHATE AT 0.015 M AND PH 5.14 IN 90/10 WATER/MEOH		
SOLVENT B:	METHANOL		
SOLVENT C:	ACETONITRILE		

SOLVENT PROGRAM

TIME	% A	% B	% C	FLOW ML/M
1	72	0	28	1
5	68	0	32	1
14	68	0	32	1
20	44	12	44	1
24	2	54	44	1
27	2	54	44	1
29	72	0	28	1
33	72	0	28	1

SAMPLE NAME:	9334 Q1 M1	RECOVERY STD:	1,3-DINITROBENZENE
WEIGHT,GMS:	2	CONCEN, PPM:	25
EXT VOL, UL:	500	VOLUME ADDED:	100
		NG ADDED:	2500
% RECOVERY:	61.53	NG FOUND:	1538.

COMPOUND	CONC PPM (1)	AREA (2)	PPM IN TISSUE MEAS (3)		CORR. (4)	RES F (5)	CT L PPM (6)
1,3-DINITROBENZENE--RS	3.08	575	0.77		1.25	5.35	0.05
2,4,6-TRINITROTOLUENE	0.29	24	0.07	***	0.12	12.05	0.05
1,3,5-TRINITROBENZENE	0.09	8.2	0.02	**	0.04	10.8	0.05
2,4-DIAMINO-6-NITROTOLUENE	0.00	0	0.00		0.00	20.83	0.18
4,6-DIAMINO-2-NITROTOLUENE	0.00	0	0.00		0.00	8	0.05
4-AMINO-2,6-DINITROTOLUENE	0.00	0	0.00		0.00	8.7	0.15
2-AMINO-4,6-DINITROTOLUENE	0.00	0	0.00		0.00	7.81	0.06
4-HYDROXYLAMINO-2,6-DNT	0.03	1.5	0.01	*	0.01	21	0.75
TRINITROBENZYL ALCOHOL	0.00	0	0.00		0.00	27.03	0.13
TRINITROBENZOIC ACID	1.19	50.1	0.30	***	0.48	23.69	0.1
TETRANITROAZOXYTOLUENE	0.13	18.6	0.03	*	0.05	6.9	0.67

NOTES: (1) CONCENTRATION IN EXTRACT; 1:1:3 MEOH:CH3CN:BUFFER
(2) PEAK AREA IN CHROMATOGRAM
(3) CONCENTRATION IN TISSUE WITH NO RECOVERY CORRECTION
(4) CONCENTRATION IN TISSUE BASED ON RECOVERY OF DNB SPIKE
(5) RESPONSE FACTOR FOR A GIVEN COMPONENT, PPB/AREA UNIT
(6) DETECTION LIMIT FOR MEASURED CONCENTRATION IN TISSUE; VALUES FOR MEASURED CONCENTRATION IN TISSUE FALLING BELOW THIS LEVEL ARE NOT RELIABLE, PROBABLY INSIGNIFICANT
* SPECTRA IS INCONCLUSIVE IN CONFIRMING THIS COMPOUND
** APPEARS TO BE AN INTERFERENCE IN THE EXTRACT
*** MAJOR INTERFERENCE FROM EXTRACT

REVIEWED BY: [signature] DATE 5-4-90

Can't confirm TNT or TN acid

Supplement to Appendix D

BASIC Program for Calculating Detection
Limits According to Hubaux and Vos (1970).

```
LIST a:detlim.bas
Syntax error

Ok
LIST '"a:\detlim.bas," lpt1
Syntax error

Ok
LIST a"a;:
1 'PROGRAM CALLED DETLIM1, HUBAUX AND VOS, ANAL CHEM 42, 849 (1970)
100 DIM X(20), Y(20), DX(20), DY(20), YC(20), YS(20), YU(20), YL(20), YP(20)
105 PRINT "TITLE FOR DETECTION LIMIT DETERMINATION": INPUT ZQ$
110 FOR I=1 TO 50
120 PRINT "AMOUNT OF SAMPLE, PPM,NG ETC, ENTER -1 WHEN FINISHED ": INPUT X(I)
130 IF X(I)<0 THEN GOTO 170
140 N=N+1
150 PRINT "SIGNAL RESULTING FROM SAMPLE, AREA,HEIGHT,ETC. ": INPUT Y(I)
160 NEXT I
170 GOSUB 1500
180 PRINT N;" PAIRS OF POINTS HAVE BEEN ENTERED"
190 PRINT
200 PRINT "WHAT % OF THE SIGNALS MAY FALL ABOVE THE CONFIDNECE INTERVAL.  THIS
IS THE % OF THE TIME YOU ARE WILLING TO RISK MAKING A WRONG DECISION SUCH AS
SAYING AN    ELEMENT IS PRESENT AT THE DET LIMIT WHEN IT IS REALLY ABSENT."
210 INPUT AP
220 PRINT "WHAT % OF THE SIGNALS MAY FALL BELOW THE CONFIDNECE INTERVAL"
230 INPUT BP
240 PA=100-AP: PB=100-BP: PZ=100-AP-BP
250 NF=N-2
260 PRINT "ENTER THE STUDENT-t VALUE FOR ";NF; " DEGREES OF FREEDOM AND"
270 PRINT PA; "% CONFIDENCE": INPUT TA
272 IF PA=PB THEN GOTO 294
280 PRINT "ENTER THE STUDENT-T VALUE FOR ";NF; " DEGREES OF FREEDOM AND"
290 PRINT PB; "% CONFIDENCE": INPUT TB
292 GOTO 300
294 TB=TA
300 PRINT
310 PRINT "YOU HAVE ENTERED THE FOLLOWING VALUES FOR CONFIDENCE EVALUATION"
320 PRINT "% CONFIDENCE THAT UPPER INTERVAL IS NOT EXCEEDED "; PA
330 PRINT "THE STUDENT-t FOR ";NF;" DEGREES OF FREEDOM IS "; TA;" AT ";PA
340 PRINT "PERCENT CONFIDENCE"
350 PRINT
360 PRINT "% CONFIDENCE THAT SIGNAL IS NOT BELOW THE LOWER INTERVAL IS "; PB
370 PRINT "THE STUDENT-t FOR ";NF;" DEGREES OF FREEDOM IS "; TB;" AT ";PB
380 PRINT "PERCENT CONFIDENCE"
390 PRINT
400 PRINT "THE OVERALL % CONFIDENCE IS "; PZ
410 PRINT
420 PRINT "THESE ARE ARBITRARY VALUES CHOSEN BY YOU.  ARE THEY OK, Y OR N?"
430 INPUT G$
440 IF G$="Y" THEN GOTO 620
450 PRINT "% OF SIGNALS WHICH MAY EXCEED UPPER INTERVAL IS "; AP
460 PRINT "ENTER NEW VALUE OR -1 IF NO CHANGE DESIRED": INPUT DM
470 IF DM<0 THEN GOTO 490
480 AP=DM: PA=100-AP: PZ=100-AP-BP
490 PRINT "THE STUDENT t-VALUE FOR THE UPPER CONFIDNECE INTERVAL IS "; TA
500 PRINT "ENTER NEW VALUE FOR STUDENT t OR -1 FOR NO CHANGE": INPUT DM
510 IF DM<0 THEN GOTO 530
520 TA=DM
530 PRINT "% OF SIGNALS WHICH MAY FALL BELOW LOWER INTERVAL IS "; BP
```

```
540 PRINT "ENTER NEW VALUE OR -1 IF NO CHANGE IS DESIRED": INPUT DM
550 IF DM<0 THEN GOTO 570
560 BP=DM: PB=100-BP: PZ=100-AP-BP
570 PRINT "THE STUDENT t-VALUE FOR THE LOWER CONFIDENCE INTERVAL IS "; TB
580 PRINT "ENTER NEW VALUE FOR STUDENT t OR -1 FOR NO CHANGE": INPUT DM
590 IF DM<0 THEN GOTO 610
600 TB=DM
610 GOTO 310
620 UX=0: UY=0
630 FOR I= 1 TO N
640 UX=UX+X(I): UY=UY+Y(I)
650 NEXT I
660 XB=UX/N: YB=UY/N
670 GOSUB 1800
680 R=(XH-XL)/XL
690 SX=0: X2=0: XY=0:  SY=0: Y2=0: YR=0: R2=0
700 FOR I=1 TO N
710 DX(I)=X(I)-XB
720 SX=SX+DX(I): X2=X2+DX(I)*DX(I)
730 DY(I)=Y(I)-YB
740 SY=SY+DY(I): Y2=Y2+DY(I)*DY(I)
750 XY=XY+DX(I)*DY(I)
760 NEXT I
770 BS=XY/X2: YR=0: R2=0
780 FOR I=1 TO N
790 YC(I)=YB+BS*DX(I)
800 YS(I)=Y(I)-YC(I): YP(I)=YS(I)*100/Y(I)
810 YR=YR+YS(I): R2=R2+YS(I)*YS(I)
820 'YC IS CALCULATED VALUE OF Y. YS IS DIFFERENCE BETWEEN CALCULATED AND
830 'MEASURED VALUE OF Y
840 NEXT I
850 S2=R2/(N-2): SD=SQR(S2)
860 Y0=YB-BS*XB
870 'Y0 IS INTERCEPT OF CALIBRATION LINE
880 'NOW CALCULATE THE UPPER AND LOWER CONFIDENCE LINES
890 FOR I = 1 TO N
900 DM=DX(I)*DX(I)/X2: DM=DM+1+(1/N)
910 DN=SQR(DM)
920 DM=DN*TA*SD: ZM=DN*TB*SD
930 DN=YB+BS*DX(I)
940 YU(I)=DN+DM
950 YL(I)=DN-ZM
960 NEXT I
970 DM=XB*XB/X2: P3=DM: DM=DM+1+(1/N)
980 DN=SQR(DM)
990 PV=DN*TA
1000 'PV IS P IN HUBAUX AND VOS, ANAL CHEM, 42, 1970, PAGE 849
1010 'YK IS MINIMUM SIGNAL THAT IS DIFFERENT THAN BACKGROUND
1020 YK=Y0+PV*SD
1030 GOSUB 2000
1040 LPRINT
1050 GOSUB 3000
1060 PRINT "IF YOU DO NOT WISH TO MAKE ANY CHANGES IN CONFIDENCE LEVELS"
1070 PRINT "OR DATA, THIS SESSION IS FINISHED. IS THAT O K , Y OR N "
1080 INPUT G$
1090 IF G$="N" THEN GOTO 170
1100 PRINT "CALCULATIONS ARE CONCLUDED"
1110 END
1500 'SUBROUTINE TO CORRECT INPUT MISTAKES OR ELIMINATE DATA
1510 PRINT "    NO.      AMOUNT/CONC     SIGNAL/AREA"
```

```
1520 FOR I=1 TO N
1530 PRINT I,X(I),Y(I)
1540 NEXT I
1550 PRINT "DO YOU WISH TO CHANGE ANY VALUES, Y OR N ": INPUT G$
1560 IF G$="N" THEN GOTO 1670
1570 PRINT "ENTER NO. OF VALUE YOU WISH TO CHANGE ": INPUT ID
1580 PRINT "CURRENT X-VALUE FOR NO. ";ID; " IS "; X(ID)
1590 PRINT "TO CHANGE, ENTER NEW VALUE; TO LEAVE THE SAME ENTER -1 ": INPUT DM
1600 IF DM=-1 THEN GOTO 1620
1610 X(ID)=DM
1620 PRINT "CURRENT Y-VALUE FOR NO. ";ID; " IS "; Y(ID)
1630 PRINT "TO CHANGE, ENTER NEW VALUE; TO LEAVE THE SAME ENTER -1 ": INPUT DM
1640 IF DM=-1 THEN GOTO 1660
1650 Y(ID)=DM
1660 GOTO 1510
1670 PRINT "DO YOU WISH TO ELEMINATE ANY POINT FROM THE CALCULATIONS, Y OR N"
1680 INPUT G$
1690 IF G$="N" THEN GOTO 1772
1700 PRINT "ENTER NO. OF POINT YOU WISH TO ELEMINATE. IF YOU DO NOT WISH TO
1710 PRINT "ELIMINATE ANY POINTS THEN ENTER 0": INPUT ID
1720 IF ID<1 THEN GOTO 1780
1730 N=N-1
1740 FOR I=ID TO N
1750 X(I)=X(I+1): Y(I)=Y(I+1)
1760 NEXT I
1770 GOTO 1510
1772 PRINT "DO YOU WISH TO ADD A POINT, Y OR N ": INPUT G$
1774 IF G$="N" THEN GOTO 1780
1776 N=N+1: PRINT "ENTER AMT/CONC (X-VALUE) FOR NLW POINT ": INPUT X(N)
1778 PRINT "ENTER SIGNAL/AREA (Y-VALUE) FOR NEW POINT ": INPUT Y(N): GOTO 1510
1780 RETURN
1800 'SUBROUTINE TO DETERMINE THE MAXIMUM AND MINIMUN VALUE OF X(I)
1810 XL=X(1)
1820 XH=X(1)
1830 FOR I=2 TO N
1840 DM=X(I)-XL
1850 IF DM>0 THEN GOTO 1870
1860 XL=X(I)
1870 DM=XH-X(I)
1880 IF DM>0 THEN GOTO 1900
1890 XH=X(I)
1900 NEXT I
1910 RETURN
2000 'SUBROUTINE TO DETERMINE DETECTION LIMIT BY SUCESSIVE APPROXIMATIONS
2010 XI=.1*XL: ID=0
2020 AX=.01*XL-XI
2030 FOR I=1 TO 1000
2040 AX=AX+XI
2050 DM=(AX-XB)
2060 DN=DM*DM/X2
2070 DM=DN+1+(1/N)
2080 DN=SQR(DM)
2090 DM=DN*SD*TB
2100 DN=YB+BS*(AX-XB)-DM
2110 A1=YK-DN
2120 IF A1<0 THEN GOTO 2150
2130 C1=AX
2140 NEXT I
2150 C2=AX
2160 ID=ID+1
```

```
2170 IF ID>2 THEN GOTO 2200
2180 XI=.05*(C2-C1): AX=C1-XI
2190 GOTO 2030
2200 XK=(C1+C2)/2
2210 'XK IS THE DETECTION LIMIT CORRESPONDING  TO XSUBD IN HABAUX AND VOS
2220 YJ=YB+BS*(XK-XB)
2230 'YJ IS THE Y-VALUE CORRESPONDING TO XK, DET LIM, ON THE CALIB LINE
2240 DM=XK-XB: DN=DM*DM: Q3=DN/X2
2250 DM=Q3+1+(1/N): DN=SQR(DM)
2260 QV=TB*DN
2270 'QV IS Q FACTOR AND Q3 IS THIRD TERM OF Q IN HABAUX AND VOS
2280 YQ=Y0+PV*SD+QV*SD: W9=(YQ-YJ)*100/YQ: WZ=ABS(W9)
2290 'YQ IS CALCULATED FROM EQ 18 OF H&V; WZ IS % DIFFERENCE BETWEEN YQ AND
2300 'YJ WHICH WAS CALCULATED  BY SUCCESSIVE APPROXIMATIONS FROM THE LOWER
2310 'CONFIDNECE CURVE
2320 RETURN
2500 'X(I) IS CALIB AMOUNTS; Y(I) IS SIGNAL RESULTING FROM X(I);
2510 'N IS NUMBER OF X/Y PAIRS; I IS RESERVED FOR INDEX VARIABLE;
2520 'G$ IS USED FOR ALL Y OR N ANSWERS; DM AND DN ARE USED TEMPORARILY
2530 'AP IS THE % OF POINTS THAT MAY FALL ABOVE UPPER CONFIDENCE LINE--ALPHA
2540 'BP IS THE % OF POINTS THAT MAY FALL BELOW LOWER CONFIDENCE LINE--BETA
2550 'PA=100-AP  % CONFIDENCE A POINT IS NOT ABOVE THE UPPER LINE
2560 'TA IS THE STUDENT-t VALUE FOR PA% CONFIDENCE AND N POINTS
2570 'PB=100-BP.  THE % CONFIDENCE A POINT IS NOT BELOW THE LOWER LINE
2580 'TB IS THE STUDENT-t VALUE FOR PB% CONFIDENCE AND N POINTS
2590 'PZ IS THE OVERALL CONFIDENCE;  PZ=100-AP-BP
2600 'UX IS SUMMATION OF X(I); UY IS THE SUMMATION OF Y(I)
2610 'XB IS THE AVERAGE X-VALUE; YB IS THE AVERAGE Y-VALUE
2620 'XL IS THE LOWEST VALUE OF X; XH IS THE HIGHEST VALUE OF X
2630 'R IS THE RANGE RATIO OF X; R=(XH-XL)/XL
2640 'DX(I) IS THE DIFFERENCE BETWEEN X(I) AND THE AVERAGE OF X
2650 'SX IS THE SUMMATION OF ALL DX(I); X2 IS THE SUMMATION OF DX(I)^2
2660 'DY(I) IS THE DIFFERENCE BETWEEN Y(I) AND THE AVERAGE OF Y
2670 'SY IS THE SUMMATION OF ALL DY(I); Y2 IS SUMMATION OF DY(I)^2
2680 'BS IS SLOPE OF CALIB LINE; Y0 IS INTERCEPT OF CALIB LINE
2690 'YC(I) ARE THE CALCULATED VALUES OF Y FOR EACH X(I)
2700 'YS(I) IS THE DIFFERENCE BETWEEN EACH OBSERVED Y AND THE CALCULATED Y
2710 'YR IS THE SUMMATION OF YS(I); R2 IS THE SUMMATION OF YS(I)^2
2720 'S2 IS THE VARIANCE OF Y; SD IS THE STANDARD DEVIATION OF Y
2730 'YU(I) IS THE VALUE OF Y ON THE UPPER LINE CORRESPONDING TO EACH X(I)
2740 'YL(I) IS THE VALUE OF Y ON THE LOWER LINE CORRESPONDING TO EACH X(I)
2750 'PV IS THE P-VALUE; P3 IS THE THIRD TERM IN THE P-VALUE EXPRESSION
2760 'QV IS THE Q-VALUE; Q3 IS THE THIRD TERM IN THE Q-VALUE EXPRESSION
2770 'C1 AND C2 ARE THE VALUES OF X COMING CLOSEST TO SATISFYING THE
2780 'EQUATION IN THE SUCCESSIVE APPROXIMATION OF THE DET LIMIT IN SUB-
2790 'ROUTINE 2100.  AX IS THE CHANGING VALUE OF X IN THE SUCCESSIVE
2800 'APPROXIMATION SCHEME AND XI IS THE INCREMENT BY WHICH AX IS CHANGED.
2810 'YK IS THE MINIMUN DETECTABLE SIGNAL; XK IS THE DETECTION LIMIT
2820 'YK IS THE VALUE CORRESPONDING TO XK ON THE CALIBRATION LINE; THIS IS
2830 'Y SUB D IN HABAUX AND VOS; YQ IS THE Y SUB D VALUE CALCULATED FROM
2840 'EQUATION 18 IN HABAUX AND VOS; WZ IS THE RESIDUAL, YJ-YQ, WHICH SHOULD
2850 'APPROACH ZERO
3000 ' SUBROUTINE TO DISPLAY OR PRINT RESULTS
3010 PRINT "SLOPE= "; BS;"  INTERCEPT= ";Y0
3020 PRINT "VARIANCE= ";S2;" STANDARD DEVIATION= "; SD
3030 PRINT "MINIMUM DETECTABLE SIGNAL IS ";YK
3040 PRINT "DETECTION LIMIT IS ";XK
3050 PRINT "P-VALUE IS ";PV; "THIRD TERM--P3 IS ";P3
3060 PRINT "Q-VALUE IS ";QV; " THIRD TERM--Q3 IS ";Q3
3070 PRINT "DET LIM SIGNAL FROM CALIB ";YJ
```

```
3080 PRINT "DET LIM SIGNAL CALCULATED FROM Q-EQUATION (18) ";YQ
3090 PRINT "PERCENT DIFFERENCE BETWEEN CALIB AND Q DET LIM SIGNALS ";WZ
3100 PRINT " AMOUNT       MEASURED       CALCULATED  MEAS-CALC     MEAS-CALC"
3110 PRINT "                SIGNAL          SIGNAL      SIGNAL       % DIFF "
3120 FOR I= 1 TO N
3130 PRINT X(I), Y(I),YC(I), YS(I), YP(I)
3140 NEXT I
3150 PRINT "CONFIDENCE LIMITS MAY BE CHANGED AND POINTS ELIMINATED;"
3160 PRINT "HOWEVER, YOU MAY CHOOSE TO PRINT THIS SET OF VALUES."
3170 PRINT "DO YOU WANT A PRINTOUT OF THIS CALCULATION, Y OR N?":INPUT G$
3180 IF G$="N" THEN GOTO 3720
3190 LPRINT
3200 LPRINT
3210 LPRINT "          LINEAR CALIBRATION WITH DECISION/DETECTION LIMITS"
3220 LPRINT
3225 LPRINT "                                                    ";ZQ$
3230 LPRINT
3240 LPRINT " CALIBRATION AMOUNTS (X) RANGED FROM ";XL;" TO ";XH
3250 LPRINT " THE RANGE RATIO FOR CALIBRATION AMOUNTS WAS ";R
3260 LPRINT
3270 LPRINT N;" PAIRS OF POINTS WERE USED WITH";NF;" DEGREES OF FREEDOM"
3280 LPRINT
3290 LPRINT "THE % CONFIDNECE THAT A VALUE DOES NOT FALL ABOVE THE UPPER"
3300 LPRINT "CONFIDENCE LINE IS ";PA;"  THE STUDENT-t WAS ";TA
3310 LPRINT
3320 LPRINT "THE % CONFIDENCE THAT A VALUE DOES NOT FALL BELOW THE LOWER"
3330 LPRINT "CONFIDENCE LINE IS ";PB;"  THE STUDENT-t WAS ";TB
3340 LPRINT
3350 LPRINT " THE OVERALL % CONFIDENCE THAT A GIVEN VALUE FALLS WITHIN"
3360 LPRINT "THE UPPER AND LOWER CONFIDENCE LINES IS ";PZ
3370 LPRINT
3380 LPRINT
3390 LPRINT "SLOPE= "; BS;"  INTERCEPT= ";Y0
3400 LPRINT
3410 LPRINT "VARIANCE= ";S2;" STANDARD DEVIATION= "; SD
3420 LPRINT
3430 LPRINT "MINIMUM DETECTABLE SIGNAL IS ";YK
3440 LPRINT
3450 LPRINT "DETECTION LIMIT IS ";XK
3460 LPRINT
3470 LPRINT "P-VALUE IS ";PV; "THIRD TERM--P3 IS ";P3
3480 LPRINT "Q-VALUE IS ";QV; " THIRD TERM--Q3 IS ";Q3
3490 LPRINT
3500 LPRINT "SIGNAL ON CALIBRATION CURVE AT CALCULATED DETECTION LIMIT ";YJ
3510 LPRINT "SIGNAL AT DETECTION LIMIT CALCULATED FROM Q-EQUATION (18) ";YQ
3520 LPRINT "DIFFERENCE BETWEEN DET LIM SIGNALS FROM CALIB AND Q-EQU ";WZ;"%"
3530 LPRINT "THIS DIFFERENCE SHOULD BE LESS THAN 0.5%"
3540 LPRINT "     ------------------------------------------------------"
3550 LPRINT
3560 LPRINT " AMOUNT         MEASURED       CALCULATED     UPPER          LOWER"
3570 LPRINT "                  SIGNAL          SIGNAL       SIGNAL         SIGNAL"
3580 LPRINT
3590 FOR I= 1 TO N
3600 LPRINT X(I), Y(I),YC(I), YU(I), YL(I)
3610 NEXT I
3620 LPRINT
3630 LPRINT "     ------------------------------------------------------"
3640 LPRINT "SOME VALUES WHICH MAY BE IMPORTANT FOR OTHER DATA EVALUATION"
3650 LPRINT
3660 LPRINT "AVE VALUE OF X IS ";XB;" SUM OF ALL X- VALUES IS ";UX
```

```
3670 LPRINT "SUM (X(I)-AVE OF X) IS ";SX;" SUM (X(I)-AVE X)^2 IS ";X2
3680 LPRINT
3690 LPRINT "AVE VALUE OF Y IS ";YB;" SUM OR ALL Y-VALUES IS ";UY
3700 LPRINT "SUM (Y(I)-AVE OF Y) IS ";SY;" SUM (Y(I)-AVE Y)^2 IS ";Y2
3710 LPRINT
3720 PRINT "DO YOU WANT TO CHANGE CONFIDENCE LEVELS OR MODIFY DATA, Y OR N?"
3730 INPUT G$
3740 IF G$="Y" THEN GOTO 170
3750 RETURN
Ok
sy
```

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